REPORT

of

THE COUNCIL FOR TOBACCO RESEARCH-U.S.A., Inc.

1985

ORGANIZATION AND POLICY

The Council for Tobacco Research-U.S.A., Inc. is the sponsoring agency of a program of research into questions of tobacco use and health. It is the outgrowth of an organization formed early in 1954 by tobacco manufacturers, growers and warehousemen. Research support has been mainly through a program of grants-in-aid supplemented by contracts for research with institutions and laboratories. The Council does not operate any research facility.

The Scientific Advisory Board to The Council meets regularly to evaluate applications for research support, judging them solely on the basis of scientific merit and relevance.

The Council awards research grants to independent scientists who are assured complete scientific freedom in conducting their studies. Grantees alone are responsible for reporting or publishing their findings in the accepted scientific manner — through medical and scientific journals and societies.

William D. Hobbs
Chairman

1985 REPORT

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THE COUNCIL FOR TOBACCO RESEARCH-U.S.A., INC.

THE COUNCIL FOR TOBACCO RESEARCH-U.S.A., INC.
900 Third Avenue, New York, N.Y. 10022

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to The Council for Tobacco Research-U.S.A., Inc.
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INTRODUCTION

This report records several noteworthy events in regard to The Council.

First, it sets a Council record for a single year because it contains abstracts of 327 published scientific documents acknowledging Council support. Most of these reports were published in 1985 while others were published less recently.

At least 2,852 reports acknowledging Council support have appeared in the literature since The Council began providing funds to independent investigators doing research in smoking and health. This indicates that The Council's ongoing research program is one of the most extensive of its kind anywhere.

One of the abstracts represents what may be another first: publication of a Council-supported study in a scientific journal in the People's Republic of China.

During 1985, William U. Gardner, Ph.D., retired from the Scientific Advisory Board which he joined in 1972; he also served subsequently as The Council's Scientific Director.

Joining the Board in 1985 was another distinguished scientist, Manfred L. Karnovsky, Ph.D., who is Harold T. White Professor of Biological Chemistry at Harvard Medical School, Boston.

Since its establishment in 1954, The Council has made available more than $100,000,000 for research by 551 scientists for 924 original projects in 290 medical schools, hospitals and research institutions.

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ABSTRACTS OF REPORTS

I. Cancer-Related Studies

PLATELET-DERIVED GROWTH FACTOR: A LINK TO MALIGNANT TRANSFORMATION

Platelet-derived growth factor (PDGF) is the major mitogen of human serum. It shares structural, functional and immunological properties with the predicted protein product of the viral oncogene (v-*sis*) of the simian sarcoma virus (SSV). Human gliblastoma, fibrosarcoma, and osteosarcoma cells, which express the proto-oncogene (c-*sis*) homolog of v-*sis*, produce and secrete mitogenic molecules that are recognized by specific PDGF antiserum. The partial sequence of c-*sis* cDNA from human osteosarcoma cells is shown to code for the carboxyterminal region of the PDGF-2 chain of the biologically active molecule of the dimeric PDGF.

Antoniades, H. N. et al.

Cancer Cells 3/Growth Factors and Transformation, Cold Spring Harbor Laboratory, 145-151, 1985.

Other support: National Institutes of Health, the American Cancer Society and the Leukemia Society of America, Inc.

From the Center for Blood Research, Boston; Department of Nutrition, Harvard School of Public Health, Boston; and the Division of Biology, California Institute of Technology, Pasadena.

MOLECULAR BIOLOGY OF CYTOCHROME P-450

The cytochrome P-450-dependent monoxygenases are widely recognized as playing a primary role in the detoxification and/or activation of xenobiotics in hepatic and extrahepatic tissues. In the experiments reported here, it is shown that pretreatment of animals with various inducing agents results in increases in the activity and specific content of particular cytochrome P-450 isozymes and may result in decreases in the specific content and activity of others. Preliminary investigations in several laboratories have shown that the increase in cytochrome P-450c, the major 3-methylcholanthrene inducible isozyme, and cytochrome P-450b, the major phenobarbital inducible isozyme, is preceeded by an increase in specific mRNA levels, suggesting a role for transcriptional regulation. Investigations are continuing into the mechanism of regulation for the induction of these two proteins.

Hines, R. N., *Bresnick, E.*, Omiecinski, C., and Levin, J.

In: Rydstrom, H., Montelius, J. and Bengtsson, M. (eds.): *Extrahepatic Drug Metabolism and Chemical Carcinogenesis*, New York: Elsevier Science Publishers B. V., 1983, pp. 419-422.

Other support: National Institutes of Health and the National Cancer Institute.

From the Eppley Institute for Research in Cancer and Allied Disease, University of Nebraska Medical Center, Omaha, and the Department of Environmental Health Sciences, University of Washington, Seattle.

DNA POLYMERASE ACTIVITY IN A REPAIR-DEFICIENT HUMAN CELL LINE

A human low-density-lipoprotein (LDL) receptor-deficient diploid fibroblast cell line (GM1915) was determined to be short patch competent (DNA polymerase-β) and long patch deficient (DNA polymerase-α) for DNA excision repair. Analysis of DNA from GM1915 cells or from W138 control cells, following trestment with a mutagen known to initiate long patch excision repair, showed that GM1915 cells exhibited decreased resynthesis of oligonucleotide segments excised during repair. When cells deficient in DNA polymerase-α activity were permeabilized to permit LDL entry, repair synthesis immediately increased. These data suggest that DNA polymerase-α is not activated by mutagen treatment in GM1915 cells and that introduction of LDL into the cells results in activation of the enzyme.

Joe, C. O., Norman, J. O., Irvin, T. R., and *Busbee, D. L.*

Biochemical and Biophysical Research Communications 128(2):754-759, 1985.

Other support: National Institutes of Health, U. S. Department of Agriculture, and the Texas Agricultural Experiment Station.

From the Department of Anatomy, and Department of Physiology and Pharmacology, College of Veterinary Medicine, Texas A & M University, College Station, and the Veterinary Toxicology and Entomology Research Laboratory, U.S. Department of Agriculture, College Station, TX.

POLYCHLORINATED BIPHENYL UPTAKE AND TRANSPORT BY LYMPH AND PLASMA COMPONENTS

The uptake and vascular transport of ingested Aroclor 1242, an isomeric mixture of polychlorinated biphenyls (PCB), was investigated in experimental animals. High concentrations of ingested PCB were found in the chylomicron fraction of thoracic duct lymph. When the lymph flow was exteriorized, PCB were not subsequently found in the vascular circulation. When lymph was not exteriorized plasma PCB concentrations reached maximal levels 6 hr after ingestion. Less than 1% of total plasma PCB was detected in cellular fractions of blood over a 10-hr period following ingestion. Chylomicrons contained 31% of total plasma PCB 30 min after ingestion, decreasing to less than 6% at 4 hr. A maximum of 10% of plasma PCB at 1 hr, and <5% at 6 hr, after ingestion was associated with very low density lipoproteins (VLDL) or low density lipoproteins (LDL). Although PCB enter the vascular circulation with the chylomicron fractions of lymph, delipoproteinated plasma contained 52% of the total PCB in blood collected 30 min after ingestion. This level increased to 78% after 2 hr and remained constant at about 80% for an additional 8-hr period. High performance liquid chromatographic examinations of delipoproteinated plasma from blood taken 6 hr after PCB ingestion showed elution of > 95% of plasma PCB to coincide with the albumin peak. Electrophoretic examinations of delipoproteinated plasma showed the association of PCB with albumin to be noncovalent. The results suggest that apolar PCB are absorbed into intestinal epithelial cells from which they are secreted into the lymphatic drainage sequestered within the apolar core of chylomicrons, that these PCB transit the thoracic duct and enter the vascular circulation within chylomicrons and are metabolized or otherwise released from chylomicrons during hepatic chylomicron clearance, and that resulting PCB or PCB derivatives circulate in association with plasma albumins.

Busbee, D.L. et al.

Proceedings of the Society for Experimental Biology and Medicine 179:116-122, 1985.

Other support: National Institutes of Health, Texas Agricultural Experimental Station, and the U. S. Department of Agriculture, College Station, TX.

From the Department of Anatomy and Departments of Physiology and Pharmacology, College of Veterinary Medicine, Texas A & M University, College Station.

ALKYLATION OF DEOXYGUANOSINE BY THE SESQUITERPENE LACTONE HYMENOXON

Hymenoxon, a toxic sesquiterpene lactone found in the ruminant forage plant *Hymenoxys odorata*, binds deoxyguanosine in a cell-free system and forms adducted guanine residues in sheep lymphocyte DNA. Mitogen-stimulated DNA synthesis in lymphocytes was inhibited by hymenoxon at concentrations greater than 100 μM. Unscheduled DNA synthesis in lymphocytes was initiated by hymenoxon concentrations exceeding 50 μM, and inhibited by concentrations above 100 μM. We describe an HPLC method which separates unmodified hymenoxon and deoxyguanosine from the hymenoxon-deoxyguanosine adduct and allows the quantitation of adducts in hymenoxon-treated cells.

Sylvia, V. L., Joe, C. O., Stipanovic, R. D., Kim, H. L., and *Busbee, D. L.*

Toxicology Letters 29:69-76, 1985.

Other support: National Institutes of Health, U. S. Department of Agriculture and the Texas Agriculture Experiment Station.

From the Department of Anatomy and Department of Physiology and Pharmacology, College of Veterinary Medicine, Texas A & M University, College Station, and the Veterinary Toxicology Research Laboratory, U. S. Department of Agriculture, College Station, TX.

UPTAKE AND VASCULAR TRANSPORT OF INGESTED AFLATOXIN

The uptake and vascular transport of gastrically instilled aflatoxin B_1 (AFB_1) was investigated in sheep. Aflatoxin uptake was compared with that of palmitate, a water-insoluble oil known to be absorbed into the intestinal lymphatic drainage which bypasses the liver to enter the peripheral vascular circulation via the thoracic duct. After instillation in animals with exteriorized thoracic duct flow, AFB_1 was detected in inferior vena cava blood within 30 min, while palmitate was not detected in vena cava blood at any time. Palmitate was detected in thoracic duct lymph after approximately 2 hr. Greater than 95% of palmitate in lymph was associated with the chylomicron fraction, while aflatoxin in either plasma or lymph was not detectably associated with any of the circulating lipoproteins. In addition, AFB_1 did not partition into plasma or lymph lipoproteins *in vitro*. Toxic lipophilic xenobiotics, such as benzo(α)pyrene and polychlorinated biphenyls, do partition into lipoproteins, are absorbed into the intestinal drainage, bypass the liver to directly enter the peripheral vascular circulation, and are not specifically hepatotoxic. These data indicate that the mode of AFB_1 absorption from the gastrointestinal system results in its immediate transport to the liver, which may contribute to AFB_1 hepatotoxicity.

Wilson, R., Ziprin, R., Ragsdale, S., and *Busbee, D.*

Toxicology Letters 159(1-2):69-76, 1985.

Other support: National Institutes of Health, U.S. Department of Agriculture, and the Texas Agriculture Experiment Station.

From the Veterinary Toxicology Research Laboratory, U.S. Department of Agriculture, College Station, TX, and the Department of Anatomy, and Department of Physiology and Pharmacology, College of Veterinary Medicine, Texas A & M University, College Station.

MITOCHONDRIA IN TUMOR CELLS: EFFECTS OF CYTOSKELETON ON DISTRIBUTION AND AS TARGETS FOR SELECTIVE KILLING

The basis for the difference in mitochondrial rhodamine 123 retention between carcinoma cells and normal epithelial cells is still under investigation. However, it is likely one of the factors involved is the higher mitochondrial membrane potential in carcinoma cells. It is of interest to note that this is the reverse of what has been observed in normal and transformed mink fibroblasts. Despite the lack of understanding of the basic mechanism, this work confirms, in a partial and preliminary way, Warburg's intuition that paying attention to mitochondria in tumor cells may ultimately help the treatment of cancer.

Chen, L. B. et al.

In: Levin, A. J. *et al.* (eds.): *The Transformed Phenotype*. Cold Spring Harbor Laboratory, 1984, pp. 75-86.

Other support: National Cancer Institute and the American Cancer Society.

From the Dana-Farber Cancer Institute and the Department of Pathology, Harvard Medical School, Boston.

LOCALIZATION OF ENDOPLASMIC RETICULUM IN LIVING AND GLUTARALDEHYDE FIXED CELLS WITH FLUORESCENT DYES

Certain fluorescent dyes, previously reported to localize mitochondria, when used at higher concentrations also localize a continuous net-like structure in both living and glutaraldehyde-fixed cells. A similar reticular structure can be detected by phase-contrast microscopy and whole-mount electron microscopy in potassium permanganate-fixed cells as well. This structure is mostly tubular, with some patchlike areas, and is likely to be the endoplasmic reticulum. The organization of the reticular structure is sensitive to colchicine and rotenone but not to cytochalasin B, taxol, monensin, the calcium ionophore A23187, 12-O-tetradecanoylphorbol 13-acetate, or hydrocortisone.

Terasaki, M., Song, J., Wong, J. R., Weiss, M. J., and *Chen, L. B.*

Cell 38:101-108, 1984.

Other support: National Cancer Institute.

From the Dana-Farber Cancer Institute and the Department of Pathology, Harvard Medical School, Boston.

MITOCHONDRIAL AND PLASMA MEMBRANE POTENTIALS CAUSE UNUSUAL ACCUMULATION AND RETENTION OF RHODAMINE 123 BY HUMAN BREAST ADENOCARCINOMA-DERIVED MCF-7 CELLS

Quantitative studies of MCF-7 cells (derived from human breast adenocarcinoma) and CV-1 cells (from normal African green monkey kidney epithelium), using the permeant cationic compound tetraphenylphosphonium (TPP), in conjunction with fluorescence microscopy using rhodamine 123 (Rh123), indicate that the mitochondrial and plasma membrane potentials affect both uptake and retention of these compounds. Under conditions that depolarize the plasma membrane, uptake and retention of TPP and Rh123, driven only by the mitochondrial membrane potential, are greater in MCF-7 than in CV-1. An ionophore that dissipates the mitochondrial membrane potential of MCF-7 cells causes them to resemble CV-1 cells by decreasing uptake and retention. Hyperpolarizing the mitochondrial membrane of CV-1 increases accumulation and prolongs retention; hyperpolarization of the plasma membrane further heightens this effect, causing the uptake of CV-1 cells to resemble that of MCF-7 cells even more closely. The greater uptake and retention by MCF-7 appear to be a consequence of elevated mitochondrial and plasma membrane potentials. The plasma membrane potential affects mitochondrial retention of TPP and Rh123, and its role in enhancing the effect of a difference in mitochondrial membrane potential is explained.

Davis, S., *et al.* (*Chen, L. B.*)

The Journal of Biological Chemistry 260:13844-13850, 1985.

From the Dana-Farber Cancer Institute and the Department of Patholbgy, Harvard Medical School, Boston.

MITOCHONDRIA IN LIVING CELLS: EFFECTS OF GROWTH FACTORS AND TUMOR PROMOTERS, ALTERATIONS IN CARCINOMA CELLS, AND TARGETS FOR THERAPY

Rhodamine 123 is useful for localizing mitochondria as well as for reflecting electrochemical gradients across mitochondria in living cells. The latter is substantiated by tetraphenylphosphorium (TPP), a widely used membrane potential probe. 12-0-tetradecanoylphorbol 13-acetate (TPA) converts filamentous mitochondria into a granular shape but does not affect the electrochemical gradient. Platelet-derived growth factor leads to an increase in mitochondrial membrane potential as well as to a clustering of mitochondria at the perinuclear region 3 hr after treatment of resting BALB/c-3T3 cells. Whereas the majority of tumor cell lines do not have altered electroc[illegible]ical gradients across mitochondria, the forms of expression between membrane potential and pH gradient can be varied. For numerous adenocarcinoma-derived cells, this gradient appears to be preferentially expressed as a membrane potential. Thus, the mitochondrial membrane potential is higher in adenocarcinoma cells than in normal epithelial cells. These carcinoma cells take up 5-fold to 20-fold more rhodamine 123, TPP or other lipophilic, cationic compounds that can reach mitochondria than do normal epithelial cells. This phenotype may be exploited for chemotherapy of adenocarcinoma cells. A lipophilic, cationic drug, dequalinium chloride, widely used for sore throat in humans, is highly toxic to carcinoma cells and exhibits a significant anticarcinoma activity in animals.

Chen, L. B. et al.

CANCER CELLS 3/Growth Factors and Transformation. Cold Spring Harbor Laboratory, 1985.

Other support: National Cancer Institute.

From the Dana-Farber Cancer Institute, New England Deaconess Hospital, Brigham and Women's Hospital, and Harvard Medical School, Boston.

INCREASED RHODAMINE 123 UPTAKE BY CARCINOMA CELLS

The total cellular content of the fluorescent mitochondrial-specific dye, rhodamine 123 (Rh-123), was quantified by butanol extraction as a function of time of exposure and dose for a variety of cell lines. These results were compared with observations made by fluorescence microscopy on dye localization and mitochondrial morphology. There appeared to be two categories of cell types based on Rh-123 uptake: those which progressively accumulate the dye, such as Ehrlich ascites tumor cells, carcinoma-derived lines MCF-7, PaCa-2, EJ, HeLa, and normal fibroblast line CCL 64; and those which appear to equilibrate with the extracellular dye within 1 h of incubation in Rh-123 (1 μg/ml) with a minimal level of uptake, such as the normal epithelial-derived lines CV-1 and MDCK and the transformed fibroblast line 64F3. Within the first category, the absolute value of uptake per cell correlated with the concentration of Rh-123 in the medium and with the period of exposure to the dye up to a point of apparent cellular saturation. The length of time required for apparent saturation depended on the cell type. In the second category, equilibration was very early, and the total uptake was a function of the extracellular concentration of Rh-123. This probably does not represent a saturation level of dye content in the non-accumulating, low uptake cell lines. Fluorescence microscopy revealed that Rh-123 localization was initially mitochondrial-specific for all the cell lines examined. Over time, alterations in mitochondrial morphology and cytoplasmic fluorescence was observed in the high uptake cell lines but not in the minimal uptake cell lines. Incubation of the high uptake HeLa cell line with the mitochondrial membrane potential inhibitor, p-trifluoromethoxyphenylhydrazone, substantially decreased Rh-123 uptake. These observations may indicate a transformation-related characteristic of carcinoma cell mitochondria. It may be possible to exploit the mechanism responsible for the progressive accumulation of Rh-123 by carcinoma-derived cell types for chemotherapeutic approaches to certain types of carcinomas.

Nadakavukaren, K. K., Nadakavukaren, J. J., and *Chen, L. B.*

Cancer Research 45:6093-6099, 1985

Other support: National Cancer Institute.

From the Dana Farber Cancer Institute, Boston, and the Department of Pathology, Harvard Medical School, Boston.

IDENTIFICATION AND QUANTIFICATION OF A MESSENGER RIBONUCLEIC ACID INDUCED BY POLYNUCLEAR AROMATIC HYDROCARBONS — USING A CLONED HUMAN CYTOCHROME P-450 GENE

The researchers have isolated four overlapping human genomic clones associated with the polynuclear aromatic hydrocarbon (PAH)-induced form of cytochrome P-450. The form of P-450 most closely associated with PAH induction has been defined as P_1-450. These four overlapping genomic clones span a total of 31.0×10^3 base pairs in

length with the coding sequence lying in the center of these clones. Translation *in vitro* of 3-methylcholanthrene-induced mRNA, selected with the human P_1-450 genomic clone, detects a protein with M_r 52000, which is immunoprecipitable by the anti-(mouse P_1-450) antibody. The isolated human P_1-450 genomic clone hybridizes to 3-methylcholanthrene-induced mRNA from monkey liver, benzanthracene and 3-methylcholanthrene-treated human mammary tumor cells (MCF-7), but not to isosafrole-treated human cells. Upon treatment with PAH there is a positive correlation between induced arylhydrocarbon hydroxylase (flavoprotein-linked monoxygenase) activity and the amount of mRNA that hybridizes to the isolated human genomic clone for P_1-450. The size of mRNA, induced from human cells and monkey liver by PAH, is around 3.3 x 10^3 base pairs, which is the same as the larger of two mRNA induced by PAH in the inbred strain of mouse (C57BL/6N). Their data also showed that the isolated DNA clone can detect a mRNA size of 3.3 x 10^3 base pairs from phytohemagglutinin-activated benzanthracene-treated human lymphocytes. Densitometer scanning indicated the presence of a 3.6-fold variation (highest-lowest) in the levels of lymphocyte P_1-450 mRNA contents among six individuals studied.

Kato, T., Ding, J-H., and *Chen, Y-T*

European Journal of Biochemistry 151:489-495, 1985.

Other support: North Carolina United Way.

From the Department of Pediatrics, Duke University Medical Center, Durham, NC.

DISPARATE DIFFERENTIATION IN HEMOPOIETIC COLONIES DERIVED FROM HUMAN PAIRED PROGENITORS

The researchers analyzed the differentiation of hemopoietic colonies derived from human paired daughter cells. Candidate progenitor cells were isolated by use of a micromanipulation technique from cultures of My-10 antigen-positive cord blood cells. Nine to 36 hours later the paired daughter cells were separated with a micromanipulator and allowed to form colonies in methylcellulose medium containing erythropoietin, phytohemagglutinin leukocyte-conditioned medium, and platelet-poor plasma. The cellular composition of the colonies was determined by differentiating all the cells of the May-Grünwald-Giemsa-stained preparation. Of a total of 75 evaluable pairs of colonies, 35 consisted of 28 types of disparate pairs revealing nonhomologous lineage combinations. Forty pairs were homologous in lineage expression. However, the proportions of the individual cell lineages were significantly different in the members of some of the homologous pairs. Some pairs revealed significant differences in colony size. These observations are similar to those reported for murine paired progenitors and are consistent with the stochastic model of huma stem cell differentiation.

Leary, A. G., Strauss, L. C., *Civin, C. I.*, and Ogawa, M.

Blood 66(2):327-331, 1985.

Other support: Veterans Administration and National Institutes of Health

From the Veterans Administration Medical Center and the Department of Medicine, Medical University of South Carolina, Charleston, and The Johns Hopkins Oncology Center, The Johns Hopkins University School of Medicine, Baltimore, MD.

STRUCTURE OF THE RAT α_1-ACID GLYCOPROTEIN GENE

The complete nucleotide sequence of the rat α_1-acid glycoprotein gene has been determined from an isolated lambda recombinant bacteriophage. Southern blot analysis and DNA sequencing indicate that there is only one gene per genome; it contains six exons and is located within a 3,200-base-pair fragment starting from a TATA box and extending to the polyadenylation signal AATAAA. Transcription starts 37 base pairs upstream from the beginning of the translation codon ATG. The TATA box (TATAAA) lies 26 base pairs upstream from this site. The gene contains several potential glucocorticoid receptor-binding sites, both inside and outside the structural gene.

Liao, Y.-C.J., Taylor, J. M., Vannice, J. L., *Clawson, G. A.*, and *Smuckler, E. A.*

Molecular and Cellular Biology 5(12):3634-3639, 1985.

From the Department of Pathology, School of Medicine, University of California, San Francisco; Gladstone Foundation; San Francisco General Hospital; and Department of Molecular Biology, Genentech, Inc., South San Francisco.

INTERMEDIATE-FILAMENT PROTEINS IN PARATHYROID GLANDS AND PARATHYROID ADENOMAS

The intermediate-filament proteins of normal, hyperplastic, and adenomatous parathyroid glands were analyzed immunohistochemically and by immunoblotting with monospecific antibodies. In both normal and adenomatous parathyroid glands, we found keratins with molecular weights of 52, 45, and 40 kilodaltons (Nos. 8, 18, and 19, respectively). Vimentin proteins could be identified only in stromal cells, while glial fibrillary acidic protein was not found. In normal parathyroid glands, neurofilament positivity was seen only in nerve axons. In five of 15 parathyroid gland adenomas some keratin-positive cells expressed neurofilament-like immunoreactivity also. In cytoskeletal extracts of one adenoma, the 200-kilodalton neurofilament protein was identified by immunoblotting. Thus, it appears that some parathyroid gland adenoma cells may acquire neurofilament proteins and coexpress cytokeratin and neurofilament polypeptide in a way comparable with that reported in certain neuroendocrine tumors.

Miettinen, M., Clark, R., Lehto, V-P., Virtanen, I., and *Damjanov, I.*

Archives of Pathology and Laboratory Medicine 109:986-989, 1985.

From the Department of Pathology and Laboratory Medicine, Hahnemann University School of Medicine, Philadelphia, and the Department of Pathology, University of Helsinki, Helsinki, Finland.

ORIGIN OF LAMININ IN THE EXTRACELLULAR MATRIX OF HUMAN TUMOR XENOGRAFTS IN NUDE MICE

Monoclonal antibodies reacting exclusively with laminin of human origin and a polyclonal antibody reacting with both murine and human laminin were used to immunohistochemically study the extracellular matrix of four human tumors grown as xenografts in nude mice: a lung carcinoma and a yolk sac carcinoma because they produced cell-associated laminin *in vitro*, and two hepatocellular carcinomas which did not produce cell-associated laminin *in vitro*. The extracellular matrix of the xenografts of

the lung carcinoma and the yolk sac carcinoma contained laminin of both human and murine origin. Xenografts of liver carcinoma contained only laminin of mouse origin. This shows that the malignant cells capable of laminin production *in vitro* contribute this glycoprotein to the extracellular matrix of the solid tumor formed by them *in vivo*.

Damjanov I. et al.

Virchows Archiv B [Cell Pathology] 49:45-52, 1985.

Other support: National Institutes of Health.

From the Hahnemann University School of Medicine and the Wistar Institute, Philadelphia, and La Jolla Cancer Research Foundation, La Jolla, CA.

REGULATORY FACTORS SPECIFIC FOR ADULT AND EMBRYONIC GLOBIN GENES MAY GOVERN THEIR EXPRESSION IN ERYTHROLEUKEMIA CELLS

In order to test if *trans*-acting regulatory factors specific for globin genes of the adult and embryonic stages of development exist in erythroid cells, transcriptionally active embryonic and adult globin genes on the same chromosome were transferred by cell fusion from the human leukemia cell K562 into phenotypically adult mouse erythroleukemia cells. Restriction-fragment-length polymorphisms of the K562 ζ embryonic globin genes were used to establish that all three copies of human chromosome 16 present in the K562 cell showed the same pattern of human globin gene expression after transfer to the mouse erythroleukemia cell. Adult (α) but not embryonic (ζ) human globin mRNA was detected in all nine of the independently derived mouse erythroleukemia hybrid cells, each of which contained human chromosome 16. Restriction endonuclease studies of the K562 α and ζ-globin genes after transfer into the mouse erythrolemia cell showed no evidence of rearrangements or deletions that could explain this loss of ζ-globin gene expression. These data suggest that regulation of globin gene expression in these erythroleukemia cells involves *trans*-acting regulatory factors specific for the adult and embryonic stages of development.

Anagnou, N. P., Yuan, T. Y., Lim, E., Helder, J., Wieder, S., Glaister, D., Marks, B., Wang, A., Colbert, D., and *Deisseroth, A.*

Blood 65(3):705-712, 1985.

Other support: University of California, San Francisco, the Veterans Administration, and the National Heart, Lung and Blood Institute.

From the University of California, San Francisco Hematology/Oncology Unit at the San Francisco Veterans Administration Medical Center.

CHARACTERIZATION OF A UNIQUE RNA INITIATED IMMEDIATELY UPSTREAM FROM HUMAN α1 GLOBIN GENE *IN VIVO* AND *IN VITRO*: POLYMERASE II-DEPENDENCE, TISSUE SPECIFICITY, AND SUBCELLULAR LOCATION

The authors have identified an abundant transcript initiated upstream from the canonical cap site of human α1 globin gene in bone marrow cells and in COS-7 cells transfected with an α1 globin gene-containing plasmid. Similar to the major α1 globin transcript, this upstream RNA is present almost exclusively in the cytoplasm of the trans-

fected COS-7 cells. It is also synthesized efficiently *in vitro* by RNA polymerase II in the nuclear extracts prepared from a Hela cell line and an erythroleukemia cell line, K562. RNAs isolated from these cell lines, however, do not contain this upstream transcript. The putative 5′ end of the α1 globin upstream RNA is mapped by primer extension to base -45, which is located in between the CCAAT and TATA boxes. The synthesis of this RNA *in vitro* and *in vivo*, and the close proximity of its 5′ end to the promoter of the α1 globin gene suggest a common mechanism regulating the transcriptional initiation of both the upstream and the major α1 globin RNAs.

Hess, J., Perez-Stable, C., *Deisseroth, A.*, and Shen, C-K. J.

Nucleic Acids Research 13(17):6059-6075, 1985.

Other support: National Institutes of Health, American Cancer Society and Veterans Administration Merit Review.

From the Department of Genetics, University of California, Davis, and the Department of Medicine, Division of Hematology and Oncology, University of California, San Francisco.

AVIAN ERYTHROBLASTOSIS VIRUS E26: NUCLEOTIDE SEQUENCE OF THE TRIPARTITE *onc* GENE AND OF THE LTR, AND ANALYSIS OF THE CELLULAR PROTOTYPE OF THE VIRAL *ets* SEQUENCE

An intact 5.7-kb provirus of the avian erythroblastosis virus E26 has been molecularly cloned for comparisons with avian myeloblastosis virus (AMV) and other avian tumor viruses. E26 and AMV transform hemopoietic cells exclusively. Both cause myeloblastosis, but E26 also causes erythroblastosis. Sequence analysis of the proviral DNA showed that: (i) The tripartite transforming gene of E26 forms a contiguous reading frame of 1046 codons, including 272 *gag*, 283 myb^E, and 491 *ets* codons. No subgenomic *ets*-specific mRNA was detected in E26-infected cells. By contrast, the *onc* gene of AMV consists almost entirely of a myb^A sequence expressed via subgenomic mRNA that extends over the 5′ and 3′ ends of myb^E. (ii) myb^E is only slightly diverged from the myb^A homolog of AMV and even less from the cellular proto-*myb* sequence with no characteristic mutation that sets apart the two viruses from proto-*myb*. (iii) The U5 region of the long terminal repeat (LTR) of E26 and AMV are colinear and differ only in scattered point mutations. The U3 region of the E26 LTR is different from that of AMV but is colinear and closely related with that of avian carcinoma virus MH2 and also with that of Prague Rous sarcoma virus (RSV), except for an unexpected 16-nucleotide substitution of 22 RSV nucleotides. Upstream of the 3′ LTR, the *c* region of E26 appears to be the same as that of RSV for 70 nucleotides and very similar to those of AMV and MH2 for about 20 to 30 nucleotides. Since the U3s of E26, MH2 and RSV are very closely related and neither MH2 nor RSV shows a particular erythroblast tropism, it is possible that the U3 does not play a critical role in the erythroblast tropism of E26. Electrophoretic size analyses of chicken DNA digested with restriction enzymes indicate that DNA fragments totaling over 50 kb hybridize with viral *ets* DNA.

Nunn, M., Weiher, H., Bullock, P., and *Duesberg, P.*

Virology 139:330-339, 1984.

Other support: National Institutes of Health.

From the Department of Molecular Biology and Virus Laboratory, University of California, Berkeley.

ACTIVATED PROTO-ONC GENES: SUFFICIENT OR NECESSARY FOR CANCER?

Proto-onc genes are normal cellular genes that are related to the transforming (onc) genes of retroviruses. Because of this relationship, these genes are now widely believed to be potential cancer genes. In some tumors, proto-onc genes are mutated or expressed more than in normal cells. Under these conditions, proto-onc genes are hypothesized to be active cancer genes in one of two possible ways: The one gene-onc cancer hypothesis suggests that one activated proto-onc gene is sufficient to cause cancer; the multigene-onc cancer hypothesis suggests that an activated proto-onc gene is a necessary, but not a sufficient, cause of cancer. However, mutated or transcriptionally activated proto-onc genes are not consistently associated with the tumors in which they are occasionally found and do not transform primary cells. Further, no set of an activated proto-onc gene and a complementary cancer gene with transforming function has yet been isolated from a tumor. Thus, there is still no proof that activated proto-onc genes are sufficient or even necessary to cause cancer.

Duesberg, P.H.

Science 228(4700):669-677, 1985.

Other support: National Institutes of Health.

From the Department of Molecular Biology, University of California, Berkeley.

MUTAGENESIS OF AVIAN CARCINOMA VIRUS MH2: ONLY ONE OF TWO POTENTIAL TRANSFORMING GENES (*δgag-myc*) TRANSFORMS FIBROBLASTS

Avian carcinoma virus MH2 contains two potential transforming genes, *Δgag-mht* and *δgag-myc*. Thus, MH2 may be a model for two-gene carcinogenesis in which transformation depends on two synergistic genes. Most other directly oncogenic viruses contain single, autonomous transforming (*onc*) genes and are models for single-gene carcinogenesis. To determine which role each potential *onc* gene of MH2 plays in oncogenesis, we have prepared deletion and frameshift mutants of each of the two MH2 genes by *in vitro* mutagenesis of cloned proviral DNA and have tested transforming function and virus production in cultured primary quail cells. We have found that *mht* deletion mutants [illegible] wild-type virus transform primary cells and that *myc* deletion and frameshift mutants do not. The morphologies of cells transformed by the *mht* deletion mutants and by wild-type MH2 [illegible] neither yet very considerably. Nevertheless, typical mutant transformed cells can often be distinguished from cells transformed by wild-type MH2. We conclude that the *δgag-myc* gene transforms primary cells by itself, without the second potential *onc* gene. This *myc*-related gene is the smallest that has direct transforming function. *Δgag-mht* is without detectable transforming function but may affect transformation by *δgag-myc*. Thus, MH2 behaves like a virus with a single *onc* gene, although it expresses two potential *onc* genes, and it appears not to be a model for two-gene carcinogenesis. Further work is necessary in order to determine whether the *Δgag-mht* gene possibly enhances oncogenic function of *δgag-myc* or has independent oncogenic function in animals.

Zhou, R. P., Kan, N., Papas, T., and *Duesberg, P.*

Proceedings of the National Academy of Sciences of the United States of America 82:6389-6393, 1985.

Other support: National Cancer Institute.

From the Department of Molecular Biology and the Virus Laboratory, University of California, Berkeley; and the Laboratory of Molecular Oncology, National Cancer Institute, Frederick, MD.

ARE ACTIVATED PROTO-*onc* GENES CANCER GENES?

The preponderance of 3T3 cell-transformation negatives among the tumors described in this paper suggests that either no genes have caused the negative tumors or that the assay failed to detect them. That only *ras* related proto-*onc* genes have been detected in human tumors signals another limitation of the 3T3 assay. Since the proto-*ras* mutations found by the 3T3 assay do not transform primary cells, it is possible that they are not relevant for tumor formation. Available data suggest that these are coincidental or consequential mutations rather than cancer causative mutations occurring in tumor cells, because the mutations are not consistently correlated with specific tumors and because in some cases they precede tumor formation and in other they evolve during tumor progression. Despite its effectiveness to transform 3T3 cells, it would follow that mutated proto-*ras* is not a dominant singular cancer gene, similar to a viral *onc* gene, and that the test is insufficient to determine whether proto-*onc* genes cause tumors in animals. The efficiency of the assay to identify cancer genes unrelated to proto-*onc* genes remains to be determined.

Duesberg, P. H. et al.

In: Neth, Gallo, Greaves, Janka (eds.): *Haemotology and Blood Transfusion* Vol. 29, *Modern Trends in Human Leukemia VI*. Springer-Verlag Berlin-Heidelberg, 1985, pp. 9-27.

Other support: National Institutes of Health.

From the Department of Molecular Biology, University of California, Berkeley; The Salk Institute, San Diego, CA; Laboratory of Molecular Oncology, National Cancer Institute, Frederick Cancer Research Facility, Frederick, MD; and Genentech, Inc., South San Francisco.

REGULATION OF EXTRAVASCULAR COAGULATION BY MICROVASCULAR PERMEA[illegible]

Extravascular coagulation is a prominent feature of such important pathological processes as cellular immunity and neoplasia and has been thought to result from procoagulants associated with the inflammatory or tumor cells peculiar to these entities. It was found that increased microvascular permeability alone is sufficient to induce equivalent extravascular coagulation in several normal tissues. The results indicate that saturating levels of procoagulant are present even in normal tissues and that microvascular permeability is a rate-limiting step in extravascular coagulation.

Dvorak, H. F. et al.

Science 227:1059-1061, 1985.

Other support: U.S. Public Health Service and Monsanto Corporation

From the Department of Pathology, Beth Israel Hospital, Harvard Medical School and Charles A. Dana Research Institute, Beth Israel Hospital, Boston.

MUTATIONAL ANALYSIS OF THE FUNCTIONAL DOMAINS OF THE Ad5-Ela GENE PRODUCTS EFFECTING CELL TRANSFORMATION

In this paper, the data described using viruses with specific mutations in the portion of the genome encoding the Ela 51 kd protein imply it is the viral gene product that is necessary to maintain the recognized properties of transformed cells. The conditionally lethal, cold-sensitive phenotype expressed by the mutated genomes, whether introduced into CREF cells by infection with virions or by transfection with intact genomes or plasmids, implies that the Ela 51 kd protein interacts with a host macromolecule or the Ela 48 kd polypeptide. The cold-sensitive phenotype may reflect a decreased binding constant at 32° C between the mutants' truncated proteins and a still unidentified host and/or viral macromolecules. Identification of the putative host component involved and the characteristics of the viral protein-host macromolecule interaction should reveal critical clues to understanding the mechanisms of viral transformation.

Babiss, L. E., *Fisher, P. B.* and Ginsberg, H. S.

In *Transfer and Expression of Eukaryotic Genes*, 247-262, 1984.

Other support: U. S. Public Health Service.

From the Department of Microbiology and Comprehensive Cancer Center, College of Physicians & Surgeons of Columbia University, New York.

CHEMICAL-VIRAL INTERACTIONS IN CELL TRANSFORMATION: ENHANCEMENT OF HUMAN ADENOVIRUS TRANSFORMATION OF CLONED RAT EMBRYO FIBROBLAST (CREF) CELLS BY ALKYLATING CARCINOGENS

A major advance in studying carcinogenesis has been the development of well defined cell culture systems which mimic *in vitro* carcinogenesis as it occurs *in vivo*. Utilizing these systems, stages analogous to initiation, promotion and progression have been identified and important insights into the molecular events involved in these processes should be forthcoming. In this review we describe some of our recent studies using the highly transformable CREF cell line to investigate the interactions between MMS and Ad5 in regulating the frequency of viral transformation and expression of the transformed phenotype. Our findings indicate that carcinogens enhance Ad5 transformation, but do not alter the pattern of viral DNA integration into cellular DNA or the qualitative expression of the transformed state. A likely hypothesis is that certain carcinogens exert their enhancing effect on DNA virus transformation by increasing the proportion of cells in an infected culture which can stably integrate viral DNA and thereby become transformed. In other cases, carcinogens may enhance DNA virus transformation by altering the expression of viral and/or cellular genes required for establishment of the transformed state.

Human T-cell leukemia virus, herpes viruses and Hepatitis B virus have been implicated in the development of specific human malignancies, but a direct link between viruses and the majority of human neoplasms has not been demonstrated. A possible reason for this apparent negative correlation may be that in most human malignancies, putative viruses cause cancer via synergistic interactions with initiating chemical

carcinogens, tumor promoters, hormones or other yet to be defined cofactors. Well-characterized cell culture systems should prove instrumental in elucidating the role of multiple-factor interactions in the etiology of human cancer, as well as lead to an understanding of the basic principles underlying the processes of tumor promotion and progression.

Hermo, H., Jr., Babiss, L. E., Liaw, W. S., Pinto, I. M., McDonald, R. J., and *Fisher, P. B.*

In: *Molecular Basis of Cancer, Part A: Macromolecular Structure, Carcinogens and Oncogenes*. New York: Alan R. Liss, Inc., 1985; pp. 489-512.

From the Department of Microbiology, Cancer Center/Institute of Cancer Research, College of Physicians & Surgeons of Columbia University, New York.

ANALYSIS OF THE REDUCED GROWTH FACTOR DEPENDENCY OF SIMIAN VIRUS 40-TRANSFORMED 3T3 CELLS

The authors have measured in a defined serum-free medium the platelet-derived growth factor (PDGF) and insulin requirements of normal Swiss 3T3 cells, simian virus 40-transformed 3T3 cells, and partial revertants of simian virus 40-transformed 3T3 cells. Swiss 3T3 cells displayed strong requirements for both PDGF and insulin. Both of these requirements were significantly diminished in simian virus 40-transformed 3T3 cells. Analysis of the PDGF and insulin requirements of the revertants indicated that the loss of either of these two growth factor requirements was not necessarily linked to the other, rather, the growth factor requirements were specifically associated with other parameters of transformation. The reacquisition of a PDGF requirement cosegregated with reversion to density-dependent growth inhibition, whereas reacquisition of a normal insulin requirement cosegregated with reversion to a normal growth dependence on calf serum. Anchorage dependence was dissociable from both growth factor requirements. The relationship between the PDGF requirement and density-dependent growth inhibition was further analyzed in normal 3T3 cells by measuring the PDGF requirement at different cell densities. At high cell densities, the requirement for PDGF became significantly greater. We suggest that at least in part the ability of transformed cells to grow to high saturation densities results from their loss of a requirement for PDGF.

Powers, S., *Fisher, P. B.*, and Pollack, R.

Molecular and Cellular Biology **4**: 1572-1576, 1984.

Other support: U. S. Public Health Service and National Institutes of Health.

From the Department of Microbiology, Cancer Center/Institute of Cancer Research, Columbia University College of Physicians & Surgeons, New York.

DEPRESSED INTERFERON SYNTHESIS IN SKIN FIBROBLASTS FROM LUNG CANCER PATIENTS

Skin fibroblast cell cultures derived from male adult lung cancer patients, an adult control population and a newborn population, were examined for their susceptibility to transformation with Kirsten murine sarcoma virus and their ability to respond to an interferon inducer (poly I-poly C). An association between sensitivity to viral

transformation and induction of interferon was observed. Cultures derived from lung cancer patients demonstrated an increased sensitivity to virus transformation and a decreased ability to respond to interferon induction as compared with age-matched controls and newborns.

Winters, A. L., Leach, M. F., Horton, E. J., and *Frankel, J. W.*

Journal of Interferon Research 5:465-470, 1985.

From the Department of Health and Rehabilitative Services, Veterans Administration Medical Center, Tampa, FL.

STUDIES ON GENE TRANSFER AND REVERSION TO UV RESISTANCE IN XERODERMA PIGMENTOSUM CELLS

The authors have examined several parameters which address the feasibility of complementing the UV-sensitive phenotype of xeroderma pigmentosum (XP) fibroblasts by gene transfer. We present a comparative study which demonstrates that relative to immortalized cells, human diploid cells are poor recipients for gene transfer. As measured by both transient and stable expression assays, diploid fibroblasts were completely refractory to DNA transfer by calcium phosphate coprecipitation and exhibited substantially reduced levels of expression following gene transfer by fusion with E. coli protoplasts. We also examined the significance of reversion of the phenotype of UV sensitivity in SV40-immortalized XP-A cell lines. In addition to confirming a previous report of reversion to wild-type levels of UV resistance at a frequency of $\simeq 10^{-7}$, we have attempted to facilitate the identification of XP-A cells complemented with genomic DNA by employing less stringent selection schemes and cotransfection of a selectable marker. Under these conditions, we observed an increased frequency of reversion and were unable to identify true transfectants.

Schultz, R. A., Barbis, D. P. and *Friedberg, E. C.*

Somatic Cell and Molecular Genetics 11(6):617-624, 1985.

Other support: U. S. Department of Energy.

From the Laboratory of Experimental Oncology, Department of Pathology, Stanford University, Stanford, CA.

PREPARATION AND CHARACTERIZATION OF MONOCLONAL ANTIBODIES AGAINST PHENOBARBITAL-INDUCIBLE CYTOCHROME P-450

Monoclonal antibodies against cytochrome P-450 were prepared from phenobarbital-induced rat liver microsomes. The immunoglobulin classes and subclasses, as well as the binding capacity to cytochrome P-450, of the different antibodies were characterized. Their specificity was verified by various techniques and seemed to correspond to a single form of cytochrome P-450, the major phenobarbital-inducible form. However, the antibodies were unable to inhibit completely the monooxygenase activities investigated. These antibodies may constitute very specific and powerful analytical tools for characterizing and quantifying cytochrome P-450 isoenzymes.

Letawe-Goujon, F., Kremers, P., Beaune, P., Paye, M, and *Gielen, J. E.*

Biochemical and Biophysical Research Communications 119(2):744-750, 1984.

Other support: Fonds de la Recherche Scientifique Médicale.

From the Laboratory of Medical Chemistry, Institute of Pathology, University of Liege, Liege, Belgium.

BENZO[a]PYRENE METABOLISM IN MOUSE LIVER: ASSOCIATION OF BOTH 7,8-EPOXIDATION AND COVALENT BINDING OF A METABOLITE OF THE 7,8 DIOL WITH THE *Ah* LOCUS

The 7,8-epoxidation of benzo[a]pyrene, and the 9,10-epoxidation of benzo[a]*trans*7,8-dihydrodiol coupled with covalent binding of the highly reactive diol-epoxide, are two key P-450-mediated reactions believed to be important in cancer initiation, mutagenesis and teratogenesis. New assays for these two reactions were developed with mouse liver microsomes. These two activities have apparent K_m values (approximately 6 μM) similar to that of aryl hydrocarbon hydroxylase activity. Twenty six individual 3-methylcholanthrene-treated Ah^b/Ah^d and Ah^d/Ah^d progeny of the (C57BL/6N) (DBA/2N) F_1 x DBA/2N backcross were studied. Both of the newly described activities appear to represent P-450 protein(s) that are responsible for aryl hydrocarbon hydroxylase activity and that are coordinately controlled by the Ah^b allele.

Van Cantfort, J., *Gielen, J. E.* and Nebert, D. W.

Biochemical Pharmacology 34(10):1821-1826, 1985.

Other support: Fonds de la Recherche Scientifique Medicale.

From the Laboratoire de Chimie Medical, Institut de Pathologie- Unite de Biochimie, Liege, Belgium, and Laboratory of Developmental Pharmacology, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD.

THE EXPRESSION OF DIFFERENT MONOOXYGENASES SUPPORTED BY CYTOCHROME P-450 IN NEONATAL RATS AND IN PRIMARY FETAL HEPATOCYTES IN CULTURE

In rat liver, the perinatal development of various monooxygenase activities follows different patterns, depending upon the reaction studied. The ontogeny of the 6β-, 7α- and 16α-testosterone hydroxylase activities differs very significantly.

Aldrin epoxidase and steroid-metabolizing monooxygenases are expressed in primary fetal rat liver cells in culture after treatment *in vitro* with dexamethasone. Testosterone is not metabolized by the control cells and is hydroxylated on the 6β and 16α positions following the addition of corticoids to the culture medium. The dose and time curves vary according to the hydroxylated position of the steroid. Aldrin epoxidase activity is nearly undetectable in the control cells but is present and is inducible by phenobarbital following treatment with the corticoid. Phenobarbital induces aldrin epoxidase in the absence of dexamethasone in the culture medium, providing that the cells are pretreated with the corticoid for 48 h. The use of antibodies against the main cytochrome P-450 species purified from adult and phenobarbital-treated rats confirms that a similar cytochrome P-450 can be induced in fetal cells in culture.

The perinatal regulation of biological events, such as the expression of the monooxygenases, can be reproduced in fetal rat liver cells in culture; such a model constitutes a unique tool for studying the biochemical mechanisms which control these phenomena.

Kremers, P., Letawe-Goujon, F., DeGraeve, J., Duvivier, J., and *Gielen, J. E.*

European Journal of Biochemistry 137:603-608, 1983.

Other support: Fonds de la Recherche Scientifique Medicale

From the Laboratory of Medical Chemistry, Institute of Pathology, University of Liege, Liege, Belgium.

CLONING AND STRUCTURE ANALYSIS OF THE RAT APOLIPOPROTEIN A-I cDNA

Apolipoprotein A-I, the major protein in mammalian high-density lipoprotein, acts as a cofactor for lecithin-cholesterol acyltransferase during the formation of cholesterol ester and as such, is thought to promote cholesterol efflux from peripheral cells to the liver. In this paper, the authors report the partial purification of rat liver apolipoprotein A-I mRNA by a polysome immunoadsorption technique and its cDNA cloning. Isolation of two overlapping cDNA clones enabled the researchers to derive the whole rat apolipoprotein A-I cDNA coding sequence. Comparison of the deduced protein sequence with its human counterpart reveals a striking homology between the prepropeptide precursors. Both mature protein amino-terminal regions are very homologous, suggesting that this particular domain could be involved in lipid-protein binding or lecithin-cholesterol acyltransferase activation.

Poncin, J. E., Martial, J. A. and *Gielen, J. E.*

European Journal of Biochemistry 140:493-498, 1984.

Other support: Ministère de la Politique Scientifique; Belgium

From the Laboratoire de Chimie Medicale, Institut de Pathologie, Universite de Liège, Belgium, and Laboratoire de Genie Genetique, Institut de Chimie, Belgium.

STRUCTURE AND REGULATION OF THE EPIDERMAL GROWTH FACTOR RECEPTOR

The epidermal growth factor (EGF) receptor is a transmembrane, glycosylated phosphoprotein with intrinsic protein-tyrosine phosphotransferase activity. The aminoterminal cell-surface part of the EGF receptor, which contains amino-linked carbohydrate, has a single EGF-binding site. The carboxyterminal cytoplasmic domain corresponds to *erb-B*, a known oncogene structurally homologous to other protein-tyrosine kinases. Self-phosphorylation of the EGF receptor, which occurs on its carboxyterminal domain via an intramolecular mechanism, enhances catalytic activity. Phosphorylation of the EGF receptor by protein kinase C suppresses its protein-tyrosine kinase activity. Because phosphatidylinositol kinase activity can be separated, protein-tyrosine kinase seems to be the only catalytic activity intrinsic to purified EGF receptors. In human epidermoid carcinoma A431 cells, the extent of amplification of the EGF-receptor gene determines the cellular content of EGF-receptor protein. The amount of EGF receptors in clonal variant A431 cells dictates their growth responses to EGF.

Gill, G. N., Bertics, P. J., Thompson, D. M. Weber, W., and Cochet, C.

Cancer Cells 3/Growth Factors and Transformation 11-18, 1985.

Other support: National Institute of Arthritis, Diabetes, Digestive and Kidney Diseases, and American Cancer Society.

From the Department of Medicine, Division of Endocrinology and Metabolism, School of Medicine, University of California at San Diego, La Jolla, CA.

RELATIONSHIP BETWEEN PRODUCTION OF EPIDERMAL GROWTH FACTOR RECEPTORS, GENE AMPLIFICATION, AND CHROMOSOME 7 TRANSLOCATION IN VARIANT A431 CELLS

Synthesis of the epidermal growth factor (EGF) receptor has been analyzed in a series of variant A431 human epidermoid carcinoma cell clones reported to contain different amounts of EGF binding sites. The amount of EGF receptor protein, quantitated by immunoaffinity chromatography, and EGF receptor mRNA, quantitated by cDNA hybridization, were closely correlated to the extent of EGF receptor gene amplification. This correlation existed in variants selected for reduced EGF receptors and in revertants from those variants with increased EGF receptors. There was also a correlation between the frequency of translocation of chromosome 7, containing the EGF receptor gene, and EGF receptor protein. These results support gene amplification as the mechanism enhancing A431 cell EGF receptor protein and determining growth responses.

Gill, G.N., et al.

Somatic Cell and Molecular Genetics 11(4):309-318, 1985.

Other support: National Institutes of Health and the American Cancer Society.

From the Department of Medicine, Division of Endocrinology and Metabolism, University of California at San Diego, School of Medicine, La Jolla, CA; Department of Molecular Biology, Keio University School of Medicine, Tokyo, Japan; Department of Cellular and Developmental Biology, University of Arizona, Tucson; and Laboratory of Molecular Biology and Virology, The Salk Institute, La Jolla.

SELF-PHOSPHORYLATION ENHANCES THE PROTEIN-TYROSINE KINASE ACTIVITY OF THE EPIDERMAL GROWTH FACTOR RECEPTOR

The effect of self-phosphorylation on the protein-tyrosine kinase activity of the epidermal growth factor receptor has been investigated using immunoaffinity-purified protein. Enzyme was first incubated for various times with excess ATP to phosphorylate it to differing extents; the ability of the enzyme to phosphorylate exogenous peptide substrates was then measured as a function of its self-phosphorylation state. Increasing self-phosphorylation to 1.3-1.8 mol of phosphate mol^{-1} of epidermal growth factor receptor enhanced protein-tyrosine kinase activity 2-3-fold. Comparison of the kinetics of protein-tyrosine kinase activity at different ATP concentrations revealed significant differences between unphosphorylated and phosphorylated enzyme. At low levels of ATP, a double reciprocal plot of the protein-tyrosine kinase activity of the unphosphorylated enzyme was hyperbolic, suggesting that ATP may act as an activator of the enzyme. At higher ATP concentrations, where greater levels of self-phosphorylation occurred during the reaction, the kinetics appeared linear and similar to those of the phosphorylated enzyme. Dose-response studies using three different peptide substrates (angiotensin II, gastrin, and a synthetic peptide corresponding to the self-phosphorylation

site in $p60^{v\text{-}src}$) showed that exogenous substrates inhibit receptor self-phosphorylation. In each case, half-maximal inhibition was observed at a peptide concentration approximately equal to the substrate's K_m. A kinetic analysis comparing peptide phosphorylation using unphosphorylated and prephosphorylated enzyme indicated that the self-phosphorylation site can act as a competitive inhibitor (alternate substrate) *versus* peptide substrates. These results suggest that self-phosphorylation of the epidermal growth factor receptor removes a competitive constraint so that exogenous substrates can be more readily phosphorylated.

Bertics, P. J. and *Gill, G. N.*

The Journal of Biological Chemistry 260(27):14642-14647, 1985.

From the Department of Medicine, Division of Endocrinology and Metabolism, University of California, San Diego, School of Medicine, La Jolla, CA.

MICROFIBRILS, ELASTIC ANCHORING COMPONENTS OF THE EXTRACELLULAR MATRIX, ARE ASSOCIATED WITH FIBRONECTIN IN THE ZONULE OF ZINN AND AORTA

Microfibrils are striated tubules that play a role in the formation of elastin fibers by providing a scaffold upon which newly synthesized elastin is deposited. Ultrastructural and staining studies also demonstrate microfibrils that terminate where elastin is sparse or absent in basal laminae, plasma membranes, and the collagenous matrix. The most striking accumulation of microfibrils is found in the zonule of Zinn, the transparent and elastic suspensory ligament of the lens, which contains no elastin. Application of immunocytochemical staining with a peroxidase-antiperoxidase (PAP) procedure demonstrates that fibronectin is associated with the microfibrils of the zonule and aorta. Aggregates of microfibrils are identical to oxytalan ("acid enduring") fibers that have been described in peridontal membranes and other sites subject to mechanical stress and they can be found in sites as disparate as the rabbit zonule, rat hepatic stroma and human cardiac papillary muscle, indicating that microfibrils are a widely distributed connective tissue element with a function that extends beyond elastogenesis; their association with fibronectin and localization suggests that they serve as an elastic anchoring component of the extracellular matrix.

Goldfischer, S., Coltoff-Schiller, B., and Goldfischer, M.

Tissue & Cell 17(4):441-450, 1985.

Other support: National Institutes of Health.

From the Department of Pathology, Albert Einstein College of Medicine, The Bronx, NY.

ALTERED METHIONINE METABOLISM AND TRANSMETHYLATION IN CANCER

Methionine metabolism and transmethylation are frequently altered in cancer cells. The alteration is often expressed as an inability of the cancer cells to grow when methionine is replaced by homocysteine in the culture medium, a condition that allows the growth of normal cells. This metabolic defect is termed methionine dependence. Methionine dependence may reflect an overall imbalance in transmethylation which results in the overmethylation of some substances and undermethylation of others within

cancer cells. Many carcinogens affect various stages of methionine/transmethylation metabolism. The ultimate effect of the alteration of methionine/transmethylation metabolism may be the disruption of the regulation of genes involved in the oncogenic process. The known protective effect of methionine against cancer may be due to prevention of altered methionine/transmethylation metabolism or compensation of the altered metabolism.

Hoffman, R. M.

Anticancer Research 5:1-30, 1985.

Other support: The National Cancer Institute and the George A. Jacobs Memorial Fund for Cancer Research.

From the Department of Pediatrics, University of California, San Diego, La Jolla.

EFFECT OF ANTIPLATELET ANTIBODY ON THE DEVELOPMENT OF PULMONARY METASTASES FOLLOWING INJECTION OF CT26 COLON ADENOCARCINOMA, LEWIS LUNG CARCINOMA, AND B16 AMELANOTIC MELANOMA TUMOR CELLS INTO MICE

Three different murine tumors, CT26 colon adenocarcinoma, Lewis lung carcinoma, and B16 amelanotic melanoma, were injected into syngeneic mice (BALB/c and C57BL/6J) to test the effect of rabbit anti-mouse platelet antibody on the development of pulmonary metastases. Antiplatelet antibody, when injected i.p., decreased the platelet count from 1.5 to 0.12 x $10^6/\mu l$ at 6 hr, which remained at this level for 24 hr. Antiplatelet antibody given 6 hr pre- and 18 hr post-i.v. injection of tumor cells decreased the mean number of CT 26 tumor nodules per lung by 57% (range, 47 to 65%) and decreased the mean nodule volume of tumor per lung by 37% (range, 0 to 71%) (124 experimental animals), when compared to the effect of nonimmune serum or irrelevant antiimmunoglobulin antibody in 136 control animals. With Lewis lung carcinoma, antiplatelet antibody decreased the mean number of tumor nodules by 62% (range, 57 to 78%) and decreased the mean nodule volume of tumors by 64% (range, 60 to 77%) using 48 experimental animals and 65 control animals. When tumor cells were given s.c., antiplatelet antibody given 6 hr pre-injection, 18 hr post-injection and every 48 hr thereafter also decreased the mean number of metastases by 42% in 14 experimental and 15 control animals. With B16 amelanotic melanoma, antiplatelet antibody given 6 hr pre- and 18 hr post-injection decreased the mean number of tumor nodules by 85% and decreased the mean nodule volume of tumors by 66% using 9 experimental and 9 control animals. Similar results were obtained when all three tumors were injected 6 hr after the injection of antiplatelet antibody. However, negative results were obtained if antiplatelet antibody was injected 6 hr after the injection of tumor cells. Since antiplatelet antibody has its maximum effect at 6 hr, it is likely that platelets play their role in the development of pulmonary metastases during the first 12 hr of tumor inoculation.

Pearlstein, E., Ambrogio, C., and *Karpatkin, S.*

Cancer Research 44:3884-3887, 1984.

From the Departments of Medicine and Pathology and Irvington House Institute, New York University Medical School, New York.

LACK OF EFFECT OF *IN VIVO* PROSTACYCLIN ON THE DEVELOPMENT OF PULMONARY METASTASES IN MICE FOLLOWING INTRAVENOUS INJECTION OF CT26 COLON CARCINOMA, LEWIS LUNG CARCINOMA, OR B16 AMELANOTIC MELANOMA CELLS

Honn *et al.* have recently reported a 93% reduction in the development of metastases of B16 amelanotic tumor cells given i.v. following a single dose of prostacyclin (PGI_2) and theophylline (100 μg each) 30 min prior to the injection of tumor cells. The present authors have been unable to reduce pulmonary metastases induced by the i.v. injection of CT26 colon adenocarcinoma, Lewis lung carcinoma, or B16 amelanotic melanoma cells with a similar regimen. Thus, PGI_2 and theophylline given prior to injection of tumor cells and 2 hr post-injection had no effect on the number or volume of pulmonary tumor nodules for CT26 cells, using 15 experimental and 14 control animals, Lewis lung cells, using 14 experimental and 13 control animals; or B16 amelanotic cells, using 26 experimental and 12 control animals. The PGI_2 used was shown to be active *in vitro* inhibiting tumor-induced platelet aggregation by all three tumors at 10^{-9} M and *in vivo* by inhibition of Lewis lung-induced thrombocytopenia at 1 hr, using 100 μg PGI_2 prior to the injection of tumor cells.

Karpatkin, S., Ambrogio, C., and Pearlstein, E.

Cancer Research 44:3880-3883, 1984.

Other support: National Institutes of Health.

From the Departments of Medicine and Pathology and Irvington House Institute, New York University Medical School, New York.

NEUROPEPTIDE K: A MAJOR TACHYKININ IN PLASMA AND TUMOR TISSUES FROM CARCINOID PATIENTS

Evidence is presented for the presence of an entire family of tachykinin-immunoreactive peptides in plasma and tumor tissues from patients with carcinoid tumors. The peptides include, in addition to substance P and neurokinin A, neurokinin B, an eledoisin-like peptide, and neuropeptide K, a 36 amino acid long tachykinin which contains neurokinin A at its C-terminus. Neuropeptide K seems to be the tachykinin which is present in highest concentrations in plasma as well as in acetic acid extracts of tumor tissues. It is highly biologically active and may therefore contribute to the clinical symptoms of carcinoid tumors.

Theodorsson-Norheim, E., Norheim, I., Oberg, K., Brodin, E., *Lundberg, J. M.*, Tatemoto, K., [illegible] P.G.

Biochemical and Biophysical Research Communications 131(1):77-83, 1985.

Other support: Nordisk Insulinfond, Swedish Society of Medicine, Karolinska Institute, Uppsala University, and Swedish Medical Research Council.

From the Department of Clinical Chemistry, Karolinska Hospital, the Department of Pharmacology and Biochemistry II, Karolinska Institute, Stockholm, and the Departments of Internal Medicine and Roentgenology, University Hospital, Uppsala, Sweden.

HEREDITARY OVARIAN CARCINOMA: BIOMARKER STUDIES

Three ovarian-cancer-prone kindreds were studied, two of which contained identical twin sisters concordant for ovarian carcinoma. In one kindred, both identical twin sisters had daughters with ovarian carcinoma. In another kindred, one of the identical twin sisters had an ovarian-cancer-affected daughter. Ovarian carcinoma showed vertical transmission in all three families in a pattern consonant with an autosomal dominant mode of inheritance. Medical-genetic survey of each family included detailed questionnaires with retrieval of primary medical and pathology documents on cancer of all anatomic sites. Putative biomarker determinations included: (1) *in vitro* hyperdiploidy in dermal monolayer cultures, and (2) lower serum levels of alpha-L-fucosidase ($\leq$ 275 IU/ml) in all cancer-affected patients and statistically significant lower levels in 50% risk individuals when compared to spouse and published controls (P=0.04 and P=0.0002, respectively). These findings are discussed in context with the eventual development of a risk factor profile which, given acceptable sensitivity and specificity, would enable identification of individuals who would be prime candidates for intensive surveillance/management programs.

Lynch, H. T. et al.

Cancer 55(2):410-415, 1985.

Other support: Nebraska Division, Fraternal Order of Eagles.

From the Creighton University School of Medicine and the Hereditary Cancer Institute, Omaha, NE; Boystown National Institute for Hearing and Speech Disorders in Children, Omaha; and Cornell University Medical College, Ithaca, NY.

FAMILIAL EMBRYONAL CARCINOMA IN A CANCER-PRONE KINDRED

Familial testicular cancer is rare. This report describes a family with an unusual cancer spectrum that included the infantile form of embryonal carcinoma of the testis in the son of a cancer-free but putative obligate gene carrier mother, and the adult form of embryonal carcinoma in this woman's maternal half-brother (their mutual mother had malignant melanoma and urinary bladder carcinoma). Hereditary syndrome designation remains elusive. Priority attention to biomarker research in families of this type for elucidation of cause and control is discussed.

Lynch, H. T. et al.

The American Journal of Medicine 78:891-896, 1985.

From the Department of Preventive Medicine/Public Health and the Department of Pathology, Creighton University School of Medicine, and the Hereditary Cancer Institute, Omaha, NE.

COLORECTAL CANCER IN A NUCLEAR FAMILY: FAMILIAL OR HEREDITARY?

Because of the high incidence of colorectal cancer, familial aggregations of this disease are common. Differentiation between etiologies contributing to familial clustering (which may have resulted either from common environmental exposure or from mere chance) and primary genetic factors may prove vexing to the physician. This report deals with the myriad problems encountered when attempting to make such

etiologic distinctions in order to provide appropriate surveillance and management, based upon tumor spectrum and natural history, for patients at increased cancer risk.

Lynch, H. T. et al.

Diseases of the Colon & Rectum 28(5):310-316, 1985.

From the Departments of Preventive Medicine/Public Health, Surgery, Pathology, and Radiology, Creighton University School of Medicine and the Hereditary Cancer Institute, Omaha, NE.

THE SARCOMA, BREAST CANCER, LUNG CANCER, AND ADRENOCORTICAL CARCINOMA SYNDROME REVISITED: CHILDHOOD CANCER

We studied two children who had rhabdomyosarcoma and glioblastoma and who were from a family with a hereditary cancer syndrome that was characterized by sarcoma, breast cancer, brain tumors, lung cancer, laryngeal carcinoma, leukemia, and adrenocortical carcinoma. The deleterious genotype has now been expressed through the fourth generation of this large kindred. The pedigree emphasizes the need for an extended history of several generations to arrive at a hereditary-syndrome diagnosis. A limited pedigree may result in nonappreciation of the genetic component. The pedigree illustrates that, in certain circumstances, the highly specific varieties of cancer may occur in children before it is expressed in the parent who carries the putative gene. Pediatricians, in evaluating the causes of childhood cancer, must be cognizant of cancer among adult relatives, since this recognition may aid in the diagnosis of those hereditary cancer syndromes that are characterized by cancer occurrence in children as well as adults.

Lynch, H. T., Katz, D. A., Bogard, P. J., and Lynch, J. F.

American Journal of Diseases of Children 139:134-136, 1985.

Other support: Nebraska Division, Fraternal Order of Eagles.

From the Departments of Preventive Medicine/Public Health, and of Pathology, Creighton University School of Medicine, and Hereditary Cancer Institute, Omaha, NE.

THE CUTANEOUS EVOLUTION OF NEVI IN A PATIENT WITH FAMILIAL, ATYPICAL, MULTIPLE-MOLE MELANOMA SYNDROME

For almost two decades we have followed a kindred with the familial, atypical, multiple-mole melanoma (FAMMM) syndrome. [illegible] the proband's 14-year-old daughter when she was age 5 years. We documented the evolution, both clinically and histologically, of the FAMMM phenotype in this girl for eight years.

Lynch, H. T. et al.

Pediatric Dermatology 2(4):289-293, 1985.

From the Departments of Preventive Medicine/Public Health and Dermatology, Creighton University School of Medicine, Omaha, NE; the Department of Internal Medicine, University of Nebraska Medical Center, Omaha; the Hereditary Cancer Institute, Omaha; the Dermatology Department, The Polyclinic, Seattle; and the Department of Pathology, Permian General Hospital, Andrews, TX.

BIOMARKER STUDIES IN HEREDITARY OVARIAN CARCINOMA

Ovarian cancer has been increasing in frequency during the past several decades, particularly in Western industrialized nations. It has the ignominious distinction of being the major cause of death from genitourinary cancer in women in the United States. A small but significant fraction of patients with ovarian cancer have family histories that are compatible with a primary genetic factor. The hereditary variant is heterogeneous but these pedigrees reveal a high predictability of cancer. Therefore, families that are prone to ovarian cancer merit the highest priority for biomarker investigations. When considering the generally poor surveillance measures available to us for detecting ovarian cancer sufficiently early to improve prognosis, the search for biomarkers of high sensitivity and specificity assumes major significance. In turn, such biomarkers may shed light on the etiology and pathogenesis of ovarian cancer in the general population and more importantly provide mechanisms for early detection and prevention of death from this tumor.

Lynch, H. T., Schuelke, G. S., and Lynch, J. F.

Cancer Detection and Prevention 8:129-134, 1985.

Other support: Nebraska Division, Fraternal Order of Eagles.

From the Department of Preventive Medicine/Public Health, Creighton University School of Medicine, and the Hereditary Cancer Institute, Omaha.

CANCER GENES, MULTIPLE PRIMARY CANCER, AND VON HIPPEL-LINDAU DISEASE

Von Hippel-Lindau disease is inherited by an autosomal dominant gene that may show marked expressive variability of cancer phenotype in certain patient families. We describe a patient with a strongly positive family history of this disease who at age 28 underwent craniotomy with removal of a cystic cerebellar hemangioblastoma; at age 48, he developed syringomyelia of the spinal cord, became quadriplegic, and had a progressive downhill course. At autopsy, hemangioblastomas of the cerebellum and spinal cord were found, as well as a left renal cell carcinoma, an oat cell carcinoma of the lung, a hepatocellular carcinoma, and an atypical thyroid adenoma. This tumor spectrum appears to be unique, although chance cannot be excluded. It is possible, however, that these findings might represent an expression of the deleterious genotype that became evident because of this patient's prolonged survival from his initial cerebellar hemangioblastoma.

Lynch, H. T., Katz, D. A., Bogard, P., and Lynch J. F.

Cancer Genetics and Cytogenetics 16:19-29, 1985.

Other support: Nebraska Division, Fraternal Order of Eagles.

From the Departments of Preventive Medicine/Public Health and of Pathology, Creighton University School of Medicine, and Hereditary Cancer Institute, Omaha, NE.

PANCREATIC CARCINOMA AND HEREDITARY NONPOLYPOSIS COLORECTAL CANCER: A FAMILY STUDY

This paper describes a kindred with vertical transmission of cancer through 5 generations which showed features of hereditary nonpolyposis colorectal cancer (HNPCC) in concert with pancreatic cancer. The proband is a 55-year old white male with verified pancreatic carcinoma. This patient, and subsequently his available relatives, filled out detailed medical-genetic questionnaires. Their signed permission forms enabled the authors to corroborate family, medical, and cancer (all anatomic sites) history through secured primary medical and pathology documents. HNPCC is becoming more frequently recognized than its dominantly inherited counterpart, familial multiple polyposis coli (FPC), a fact which has contributed to the recent increased interest in this disease (Lynch *et al*, 1981). There are at least two forms of HNPCC: (1) hereditary site-specific colonic cancer, referred to as Lynch syndrome I; and (2) the Cancer Family Syndrome, referred to as Lynch syndrome II (Boland and Troncale, 1984). In both disorders, one finds multiple primary cancers of the colon with an excess of involvement of the proximal colon. Lynch syndrome II includes cancer of other anatomic sites, particularly the endometrium and ovary and possibly the pancreas. Genetic heterogeneity with respect to variation in tumor spectrum has become increasingly more evident in HNPCC (Lynch *et al*., 1982). Neuroblastoma, a lesion more characteristic of childhood, in the proband's son at age 22 is puzzling. There is a need for more biomarker and pedigree studies with documentation of cancer of all anatomic sites in HNPCC kindreds (Danes and Lynch, 1982).

Lynch, H. T. et al.

British Journal of Cancer 52:271-273, 1985.

From the Department of Preventive Medicine/Public Health, Department of Internal Medicine, Division of Gastroenterology, Creighton University School of Medicine and the Hereditary Cancer Institute, Omaha; and The McGreevy Clinic, Sioux Falls, SD.

FAMILIAL MESOTHELIOMA: REVIEW AND FAMILY STUDY

To date, with few exceptions, primary attention to the etiology of malignant mesothelioma has been focused on environmental factors. However, several reports of familial aggregations of mesothelioma strongly support the supposition that host factors, in concert with environmental exposure, particularly asbestos, may contribute etiologically to an as yet unknown fraction of occurrences of this disease. However, in evaluation of familial mesothelioma, it is important to consider the possibility of household exposure to asbestos. We report a family in which two brothers with prolonged occupational asbestos exposure manifested malignant pleural mesotheliomas of similar histology.

Lynch, H. T., Katz, D., and Markvicka, S. E.

Cancer Genetics and Cytogenetics 15:25-35, 1985.

From the Departments of Preventive Medicine/Public Health and Pathology, Creighton University School of Medicine, and the Institute for Familial Cancer Management and Control, Omaha, NE.

ALVEOLAR MACROPHAGE FUNCTION AND INFLAMMATORY STIMULI IN SMOKERS WITH AND WITHOUT OBSTRUCTIVE LUNG DISEASE

To explore possible cofactors in the development of chronic obstructive pulmonary disease (COPD) in smokers, the authors performed bronchoalveolar lavage in 6 smokers with normal pulmonary function, 6 smokers with COPD ($FEV_1/FVC \leq 65\%$) matched for smoking history and age, and 9 age-matched nonsmoking control subjects. Elastase release by macrophages from smokers with COPD was significantly higher ($p \leq 0.016$) than was elastase release by macrophages from normal smokers. There were no differences between chemoattractiveness of alveolar macrophage supernatants for one person's polymorphonuclear leukocytes among the groups of smokers and there was no detectable C5/C5a in these supernatants (limit of detection of C5a greater than 1 ng/ml). There were no significant differences in numbers or species of bacteria in aerobically and anaerobically cultured bronchial brushings. There was no difference in alveolar macrophage superoxide anion release with particulate or membrane-perturbing stimuli for the smokers. Alveolar macrophages from the 3 groups of subjects had similar limited microbicidal ability for the obligate intracellular protozoan, *Toxoplasma gondii* and similar numbers of elastase receptors and affinity for elastase.

McLeod, R., Mack, D. G., McLeod, E. G., *Campbell, E. J.*, and Estes, R.G.

American Review of Respiratory Disease 131:377-384, 1985.

Other support: The Tasner and Kenzer Foundations and the Michael Reese Medical Research Institute Council, the National Institute of Allergy and Infectious Diseases, the National Heart, Lung and Blood Institute, and the Research, Education and Teaching in Pulmonary Medicine Fund of Michael Reese Medical Center, Chicago.

From the Department of Medicine, Michael Reese Hospital, Chicago; the University of Chicago Pritzker School of Medicine; The Jewish Hospital at Washington University Medical Center, St. Louis.

EXPERIMENTAL PULMONARY INFLAMMATORY INJURY IN THE MONKEY

Inflammatory pulmonary injury was induced in *Macacca mulatta* rhesus monkeys by the intrabronchial instillation of the formylated peptide norleu-leu-phe (FNLP) or phorbol myristate acetate (PMA). Indicators of pulmonary injury included an increase in mean protein content of bronchoalveolar lavage (BAL) fluid from 0.51 mg/ml in untreated animals to 3.74 mg/ml and 6.64 mg/ml in FNLP- and PMA-treated animals, respectively, the appearance of a diffuse pulmonary infiltrate in chest roentgenograms, and histologic evidence of a predominantly neutrophilic leukocytic infiltration.

Concomitant with the appearance of pulmonary injury was the generation of proteases and oxidants in the BAL fluids. Neutrophil elastase, bound to α_1-protease inhibitor was found to increase from 0.47 μg/ml in untreated monkeys to 0.99 μg/ml in FNLP-treated animals and 1.23 μg/ml in monkeys receiving PMA. Radioiodinated human prekallikrein, instilled for 2 min into the inflammatory site and retrieved by lavaging, was found to have undergone proteolytic cleavage; this cleavage was not consis-

tently inhibitable with the inclusion of antibody to elastase. BAL fluids were shown to contain an amidolytic activity when tested on the synthetic substrate H-D-pro-phe-arg-pNA. This activity was partially inhibitable with known inhibitors of active Hageman factor and kallikrein. β-Glucuronidase levels in the BAL fluids increased from 0.85 U/ml to 4.36 U/ml and 8.25 U/ml in FNLP- and PMA-treated animals, respectively. Myeloperoxidase (MPO) levels also increased from 1.37 OD U/ml min to 16.59 and 30.47 OD U/ml min in the same groups of animals.

Oxidant generation was also assessed in several different ways. The specific activity of the oxidant-sensitive inhibitor α_1-PI recovered in the BAL fluid decreased from 0.80 in control samples to 0.57 and 0.65 in FNLP- and PMA-treated animals. That this inactivation was due to oxidant injury of the molecule was confirmed by the return to full activity of four of five BAL samples after their incubation with the reducing agent dithiothreitol in the presence of methionine sulfoxide peptide reductase. The specific activity of catalase in the BAL fluids of animals given 3-amino, 1,2,4 triazole (AT) 1 h before lavaging showed drops from 0.97 in untreated monkeys to 0.04 in FNLP-treated and 0.49 in PMA-treated monkeys. MPO levels also fell in the AT-treated injured animals from 16.59 to 0.85 Δ OD/min ml in FNLP animals in the absence and presence of AT, and 30.47 to 0.60 Δ OD/min ml in PMA-treated animals. Inhibition of MPO by AT was shown *in vitro* to be hydrogen peroxide dependent. Total glutathione levels in the BAL fluids did not change appreciably after FNLP or PMA treatment.

These studies present substantial evidence of the generation of both proteases and oxidants during the establishment of acute pulmonary inflammatory injury in an experimental primate model.

Revak, S. D., *Cochrane, C. G. et al.*

Journal of Clinical Investigation 76:1182-1192, 1985.

Other support: U. S. Public Health Service and Office of Naval Research.

From the Department of Immunology, Scripps Clinic and Research Foundation, La Jolla, CA and the Department of Surgery, Harborview Medical Center, Seattle.

ISOLATION OF TROPOELASTIN a FROM LATHYRITIC CHICK AORTAE

Tropoelastin a was isolated from lathyritic chick aortae by using severe denaturing conditions for the initial extraction. The amino acid composition of this new species of tropoelastin is elastin-like in its high proportion of proline, glycine, alanine and valine. However, it differs from authentic tropoelastin b in containing a higher percentage of polar amino acids and cysteine residues. In addition, the amount of proline hydroxylation is 3 times higher than that found in chick tropoelastin b.

Rich, C. B. and *Foster, J. A.*

Biochemistry Journal 217:581-584, 1984.

Other support: National Institutes of Health.

From the Department of Biology, Syracuse University, Syracuse, NY.

EFFECT OF HYDROXYL RADICAL SCAVENGING ON ENDOTOXIN-INDUCED LUNG INJURY

The release of oxygen radicals, in particular the hydroxyl radical, from sequestered neutrophils produces acute lung injury after a number of insults. Our purpose was to determine whether hydroxyl radical OH· is responsible for the lung injury from endotoxin characterized by (1) pulmonary leukostasis, (2) increased thromboxane production leading to pulmonary hypertension and hypoxia, and (3) increased protein permeability. This hypothesis was tested by infusion of a selective OH· scavenger, dimethyl thiourea (0.75 gm/kg), into unanesthetized sheep before endotoxin and comparing the response to that seen with endotoxin alone. Pulmonary vascular integrity was measured by the use of lung lymph flow, Q_L, lymph protein transport, Thromboxane A_2 was measured as TxB_2, and prostacyclin as 6-keto-PGF_{1a}. We found no difference in the degree of leukopenia and hypoxia after endotoxin or the levels of TxB_2, 6-keto-PGF_{1a} and pulmonary hypertension with dimethyl thiourea, compared with endotoxin alone. The permeability injury was also identical, with a two-fold to three-fold increase in protein-rich lymph seen in both groups. It appears that OH· does not play a major causative role in either phase of endotoxin lung injury.

Wong, C., *Fox, R.*, and Demling, R. H.

Surgery 97(3):300-306, 1985.

Other support: National Institutes of Health.

From the Longwood Area Trauma Center, Beth Israel Hospital, Harvard Medical School, and Brigham and Women's Hospitals, and the Children's Hospital, Boston.

PROSTAGLANDINS AND INTRACELLULAR CYCLIC AMP IN RESPIRATORY SECRETORY CELLS

Prostaglandins are known to affect ion transport and mucus secretion in the trachea, and at least part of their effect is thought to be mediated by cyclic AMP. Because no prostaglandin receptor assay is currently available, we have used an immunocytochemical probe for the intracellular localization of cyclic AMP to identify those specific cell types in dog and cat trachea that respond to prostaglandins. Using tracheal explants similar to those used by other investigators for *in vitro* studies of ion transport and glycoprotein secretion, we examined the effect of endogenous and exogenous prostaglandins on immunoreactive cyclic AMP. Endogenous prostaglandins, which may be secreted in response to minor distortion of tissue membranes, stimulated immunoreactive cyclic AMP in ciliated epithelial cells and in both serous and mucous submucosal gland cells. This was not observed in tissues assayed 60 min after dissection or dissected in the presence of indomethacin or of BW755C. Exogenous prostaglandins increased cyclic AMP in these same cell types. We conclude that prostaglandins stimulate cyclic AMP in specific cell types in the trachea, and that endogenous prostaglandins play a major role in many *in vitro* preparations.

Lazarus, S. C. Basbaum, C. B. and *Gold, W. M.*

American Review of Respiratory Disease 130:262-266, 1984.

Other support: National Heart, Lung and Blood Institute.

From the Cardiovascular Research Institute and the Departments of Medicine and Anatomy, University of California, San Francisco.

AIRWAY RESPONSIVENESS TO INHALED ANTIGEN, HISTAMINE, AND METHACHOLINE IN INBRED, RAGWEED-SENSITIZED DOGS

The authors studied the responses to antigen in animals selected from a colony of inbred dogs sensitized to specific allergens to determine if they had characteristics similar to those of human asthmatics. They were immunized with ragweed and grass pollen extracts (10 μg in alum) immediately after routine vaccination with attenuated live virus (distemper and hepatitis) and killed bacteria (Leptospira) at 4, 8, and 12 wk of age. Subsequently, ragweed and grass injections were repeated every 2 months. Immunized dogs made specific IgE-antibodies in serum averaging 3 to 4 times that of control animals (no immunization with pollen or vaccine). They showed positive skin responses to the injection of ragweed pollen extract, whereas control dogs did not respond to ragweed pollen by quantitative skin test or inhalation challenge. In immunized dogs under barbiturate anesthesia, air-flow resistance of the total respiratory system increased from 0.60 ± 0.07 (mean ± SEM) before to 12.6 ± 3.4 cm H_2O/lps 5 min after the start of antigen aerosol; respiratory resistance remained increased for 20 min and was associated with hypoxemia and increased arterial plasma histamine. In addition, airway responsiveness to both inhaled histamine and methacholine was greater in immunized dogs than in nonimmunized dogs of comparable age. Airway responses to each agonist were highly reproducible on repeated testing. These results indicate that physiologic responses to antigen by inbred, ragweed-sensitized dogs resemble human asthma closely and that these dogs appear suitable for a variety of experimental studies of asthma with respect to pathogenesis, diagnosis, prevention, and treatment.

Mapp, C., Hartiala, J., Frick, O. L. Shields, R. L., and *Gold, W. M.*

American Review of Respiratory Disease 132:292-298, 1985.

Other support: National Lung and Blood Institute, National Institute of Allergy and Infectious Diseases, and the Northern California Allergy Society.

From the Cardiovascular Research Institute and Departments of Medicine and Pediatrics, University of California, San Francisco, and the Animal Resources Services Facility of the School of Veterinary Medicine, University of California, Davis.

CHARACTERIZATION OF PURIFIED DOG MASTOCYTOMA CELLS: AUTONOMIC MEMBRANE RECEPTORS AND PHARMACOLOGIC MODULATION OF HISTAMINE RELEASE

There is conflicting evidence as to which autonomic receptors mast cells possess and whether the receptors are capable of modulating mediator release. The authors have studied dog mastocytoma cells because they are available in large numbers in a relatively pure form, unlike normal dog mast cells. Mastocytoma nodules from a dog were excised and disaggregated with collagenase to provide a cell suspension of mastocytoma cells of ≥ 92% purity. The presence of autonomic receptors was assessed by both radioligand binding assays and by evaluating pharmacologic modulation of mediator release. In the radioligand binding assays, beta-adrenergic receptors were estimated by [^{3}H]prazosin binding and cholinergic receptors by [^{3}H]quinuclidinyl benzilate binding. Nonspecific binding was determined in each case by incubation in the presence of the specific

antagonists propranolol, phentolamine, and atropine, respectively. The effect of autonomic agonists on immunologic and nonimmunologic histamine release was examined using the beta-adrenergic agonists isoproterenol and terbutaline, the alpha-adrenergic agonist phenylephrine with and without propranolol, and the cholinergic agonist acetylcholine. Dose-response curves were constructed both for the autonomic agonists and the histamine-releasing agents. Results from the radioligand binding and the pharmacologic studies were concordant. These dog mastocytoma cells had a high density of beta-receptors (21,500 ± 3,300; mean ± SE beta-receptors/cell, n=5) of which the predominant subtype appeared to be $beta_2$. No evidence was found for the presence of alpha-adrenergic or cholinergic receptors either by direct receptor binding or by their actions on histamine release.

Phillips, M. J., Barnes, P. J., and *Gold, W. M.*

American Review of Respiratory Disease 132:1019-1026, 1985.

Other support: National Heart, Lung and Blood Institute, the Strobel Medical Research Fund of the American Lung Association of San Francisco, the University of California Research Evaluation and Allocation Committee, and the California Research and Medical Education Fund of the American Lung Association of California.

From the Cardiovascular Research Institute and Department of Medicine, University of California, San Francisco.

NICOTINE-INDUCED RESPIRATORY EFFECTS OF CIGARETTE SMOKE IN DOGS

The authors report that nicotine is responsible for both a blood-borne stimulation of the respiratory center and a direct effect on intrathoracic airway tone in dogs. They introduced cigarette smoke into the lungs of donor dogs and injected arterial blood obtained from them into the circulation of recipient dogs to show that a blood-borne material increased breathing and airway smooth muscle tone. Smoke from cigarettes containing 2.64 mg of nicotine was effective; that from cigarettes containing 0.42 mg of nicotine was not. Nicotine, in doses comparable to the amounts absorbed from smoke, also increased breathing and tracheal smooth muscle tension when injected into the vertebral circulation of recipient dogs. Finally, blockade of nicotine receptors in the central nervous system and in the airway parasympathetic ganglia inhibited the effects of inhaled cigarette smoke and intravenous nicotine on the respiratory center and on bronchomotor tones. The authors concluded that nicotine absorbed from cigarette smoke is the main cause of cigarette smoke-induced bronchoconstriction. It caused central respiratory stimulation, resulting in increased breathing and airway smooth muscle tension, and had a direct effect on airway parasympathetic ganglia as well.

Hartiala, J. J., Mapp, C., Mitchell, R. A., and *Gold, W. M.*

Journal of Applied Physiology 59(1):64-71, 1985.

Other support: National Heart, Lung, and Blood Institute.

From the Cardiovascular Research Institute and Departments of Medicine and Physiology, University of California, San Francisco.

AN ULTRASTRUCTURAL ANALYSIS OF DOG MASTOCYTOMA CELLS AND NORMAL MAST CELLS

A well-differentiated dog mastocytoma was characterized ultrastructurally using morphometric, histochemical, and biochemical methods. The ultrastructure of cells in the intact tumor was compared to the morphology of cells disaggregated from the tumor and cultured for periods as long as 4 weeks and to normal dog connective tissue mast cells. Most of the tumor cells contained histamine (mean = 5.81 pg/cell), demonstrated chloroacetate esterase activity histochemically, stained metachromatically with toluidine blue, and were similar in ultrastructure to normal dog mast cells. The proportion of mast cells in this tumor averaged 67%; eosinophils, fibroblasts, plasma cells, and macrophages also were present. The mean diameter of mast cells (12.79 μm) and the mean diameter of their cytoplasmic granules (473 nm) were similar to those reported for mast cells and mastocytoma cells from various species. The heterogeneity in appearance of the mastocytoma granules was consistent with a variable degree of granule maturation. After disaggregation or periods of culture ranging from 2 days to 4 weeks, the mean granule diameters were 15% larger than those measured in the intact mastocytoma cells, though other morphologic features remained unchanged. Although the cells retained their distinct morphological features for at least 4 weeks, some of their physiological responses were lost after 1 week in culture. This study showed that dog mastocytomas can be a source of a large, relatively homogeneous population of cells that are useful for elucidating some of the structural and functional properties of mast cells.

Calonico, L. D., Phillips, M. J., McDonald, D. M., and *Gold, W. M.*

The Anatomic Record 212:399-407, 1985.

Other support: National Heart, Lung and Blood Institute, the Strobel Medical Research Fund of the American Lung Association of San Francisco, and the University of California Research and Medical Education Fund of the American Lung Association of California.

From the Cardiovascular Institute and Departments of Anatomy and Medicine, University of California, San Francisco.

IMMUNOLOGIC CHALLENGE AND EPITHELIAL ION TRANSPORT IN CANINE TRACHEA

In this study the authors used measured changes in short-circuit current to reflect ion transport across the canine tracheal epithelium and have shown that mediators released from lung fragments by immunologic challenge stimulate ion transport in the trachea. The presence of histamine in the supernatants, although the concentration is too low to account for the short-circuit current effect, indicates that mast cell degranulation has occurred. Particularly, the observation that the increase in short-circuit current can be blocked by pretreatment of the epithelial tissue with the cyclooxygenase inhibitor indomethacin suggests that the mediator effect on short-circuit current is *indirect* and is produced by activating the cyclooxygenase pathway in the epithelium.

Lazarus, S. C., Leikauf, G. D., McCabe, L. J., Paige, K., Chung, K. F., Nadel, J. A., and *Gold, W. M.*

Chest 287S:188S-189S, 1985.

Other support: National Heart, Lung and Blood Institute, the California Research and Medical Education Fund of the American Lung Association of California, and the Research Evaluation and Allocation Committee of the University of California School of Medicine, San Francisco.

From the Cardiovascular Research Institute and Department of Medicine, University of California, San Francisco.

PROTECTIVE REFLEXES AND VASCULAR EFFECTS IN THE NASAL MUCOSA ELICITED BY ACTIVATION OF CAPSAICIN-SENSITIVE SUBSTANCE P-IMMUNOREACTIVE TRIGEMINAL NEURONS

The aim of the present investigation was to study the structure and functional characteristics of the sensory pathways which provide protective reflexes in the nasal mucosa in various experimental animals. The use of capsaicin desensitization as a possible treatment against nasal irritation has also been evaluated. The following specific aspects were studied.

(1) The origin and distribution of SP-IR nerve fibres in the nasal mucosa were investigated in several species, including man. Also the effect of systemic capsaicin pretreatment on SP-containing neurons was studied.

(2) The effect of antidromic trigeminal and parasympathetic nerve stimulation on cat nasal blood flow was compared with the response seen following local infusions of SP, VIP and capsaicin.

(3) The effects of antidromic trigeminal nerve stimulation, local application of SP or capsaicin on plasma extravasation in the nasal mucosa were studied in control and capsaicin-pretreated rats.

(4) The effect of cigarette smoke and its various components on nasal mucosal permeability was studied in the rat. Additionally, the sensory mechanisms underlying the avoidance behavior (nose-wipings) induced by cigarette smoke was investigated in the guinea-pig.

(5) The mechanisms underlying sneezing responses to both chemical irritants and tactile stimulation were studied following capsaicin pretreatment.

(6) The acute and long-term effects of local systemic capsaicin pretreatment and cryosurgery on the nasal mucosa were studied. The experiments examined the inhibition of nasal irritation, loss of SP-IR neurons, neurogenic plasma extravasation, local tissue damage and systemic effects following these treatments.

(7) The importance of capsaicin-sensitive sensory nerves for the cardiovascular responses induced by local application of nicotine and capsaicin to the nasal mucosa was also investigated.

Lundblad, L. *(Lundberg, J. M.)*

Acta Physiologica Scandinavica Supplement 529:1-42, 1984.

Other support: The Swedish Medical Research Council, the Swedish Tobacco Company, the Astra Foundation and the Karolinska Institute.

From the Department of Pharmacology, Karolinska Institute, and the Department of Oto-rhino-laryngology, Karolinska Hospital, Stockholm, Sweden.

EVIDENCE FOR SUBSTANCE P-IMMUNOREACTIVE SPINAL AFFERENTS THAT MEDIATE BRONCHOCONSTRICTION

The origin and functional role of capsaicin-sensitive substance P-(SP-) immunoreactive (IR) nerve fibres in the lower airways were studied in the guinea-pig. Stellatectomy caused a significant reduction of SP-IR in the lung and pulmonary artery. Immunohistochemical analysis, however, did not reveal any clear-cut change in the number and distribution of SP-IR fibres in the lung of these animals. After combined stellatectomy plus local capsaicin treatment of the vagal nerves, most SP-IR nerves disappeared in the lower airways. The bronchoconstriction induced by capsaicin was significantly reduced after stellatectomy and abolished after stellatectomy plus capsaicin pretreatment of the vagal nerves. Ether inhalation caused bronchoconstriction, which was not influenced by stellatectomy but markedly reduced by combined capsaicin treatment of vagal nerves and stellatectomy. Stellate ganglion stimulation in animals that had been chemically sympathetctomized by 6-OH-dopamine caused bronchoconstriction, which was resistant to cholinergic or adrenergic receptor blockade. This response was absent after systemic capsaicin pretreatment, suggesting that it was due to antidromic stimulation of afferent fibres traversing the stellate ganglion. In conclusion, the present data suggest that the lower airways receive SP-IR capsaicin-sensitive C-fibre afferents of both vagal and spinal origin. These sensory fibres seem to have branches both within the bronchial smooth muscle and around blood vessels.

Saria, A., Martling, C.-R., Dalsgaard, C.-J., and *Lundberg, J. M.*

Acta Physiologica Scandinavica 125:407-414, 1985.

Other support: The Swedish Tobacco Company, the Gustaf V. Foundation, the Astra Foundation, the Petrus and Augusta Hedlunds Fonds, the Karolinska Institute, Austrian Scientific Research Funds, and the Swedish Medical Research Council.

From the Department of Experimental and Clinical Pharmacology, University of Graz, Graz, Austria; the Departments of Pharmacology and Anatomy, Karolinska Institute; and the Department of Anesthesiology, Karolinska Hospital, Stockholm, Sweden.

SEQUENCE OF PATHOLOGIC CHANGES IN THE AIRWAY MUCOSA OF GUINEA PIGS DURING OZONE-INDUCED BRONCHIAL HYPERREACTIVITY

The authors assessed the nature and progression of airway mucosal disease and muscarinic bronchial reactivity in guinea pigs studied in groups of 4 at 2h, 6h, 14h, 1 day, 2 days, or 4 days after ozone exposure (3.0 ppm for 2h), and in 1 control group. Muscarinic reactivity was determined by measuring specific airway resistance as a function of increasing doses of intravenous acetylcholine in 31 intact, unanesthetized, spontaneously breathing animals. After testing, each group was killed to obtain tracheal tissue for light microscopic examination. We found that airway hyperreactivity to acetylcholine occurred in 96% of the animals exposed to ozone. Its degree at 2h was substantial. Complete remission was not observed until the fourth day. In association with the acute bronchial hyperreactivity found at 2h, a marked decrease in airway mucosal goblet cells and an increase in mucosal mast cells occurred. Neutrophilic infiltration occurred later and lasted longer, despite remission of the hyperreactivity.

Our results indicate that acute, ozone-induced bronchial hyperreactivity is related to signs of airway mucosal injury and mast cell infiltration. After this early phase of airway damage, neutrophilic infiltration occurs and persists, suggesting that it is a consequence of the damage rather than a cause of the increased airway reactivity after ozone exposure.

Murlas, C. and Roum, J. H.

American Review of Respiratory Disease 131:314-320, 1985.

Other support: National Heart, Lung and Blood Institute.

From the Department of Medicine, University of Cincinnati, Cincinnati, OH.

OZONE-INDUCED CHANGES IN MUSCARINIC BRONCHIAL REACTIVITY BY DIFFERENT TESTING METHODS

We examined the effect of ozone (0_3) on muscarinic bronchial reactivity in the guinea pig and compared reactivity determined by two different routes of agonist delivery. Reactivity before and from 4h to 2 days after 0_3 exposure (3.0 ppm, 2h) was determined by measuring specific airway resistance upon administration of intravenous acetylcholine and/or aerosolized methacholine challenge in 34 unanesthetized, spontaneously breathing animals. Before exposure, we observed more gradual and reproducible results to intravenous agonist. After exposure, hyperreactvity to parenteral agonist occurred consistently, but not to inhaled agonist. Hyperreactivity demonstrable by either route was similar in magnitude and time course within 14h of exposure. Two days later, hyperreactivity to inhaled agonist had remitted; that to intravenous drug persisted. Our results indicate that variability in the occurrence and time course of 0_3-induced hyperreactivity to inhaled agonist may be a consequence of the technique employed. The consistent occurrence of hyperreactivity after 0_3 to parenteral agonist suggests mechanisms other than airway mucosal hyperpermeability are responsible for this hyperreactivity.

Roum, J. H. and *Murlas, C.*

Journal of Applied Physiology 57(6):1783-1789, 1984.

Other support: National Heart, Lung and Blood Institute, National Institutes of Health and American Lung Association.

From the Departments of Medicine, Physiology and Biophysics, University of California, Irvine, and Department of Medicine, University of Cincinnati, Cincinnati, OH.

OZONE-INDUCED BRONCHIAL HYPERREACTIVITY IN GUINEA PIGS IS ABOLISHED BY BW 755C OR FPL 55712 BUT NOT BY INDOMETHACIN

We investigated the effects of BW 755C, an inhibitor of both the cyclooxygenase and lipoxygenase pathways of arachidonic acid metabolism; FPL 55712, a selective antagonist of slow-reacting substance of anaphylaxis; and indomethacin, a cyclooxygenase inhibitor, on bronchial reactivity after ozone exposure. Guinea pigs in groups of 5 were treated with BW 755C (10 mg/kg given intravenously), FPL 55712 (5 mg/kg given intravenously), or indomethacin (30 mg/kg given intraperitoneally) and studied before and 30 min after a 15-min exposure to 3.0 ppm ozone. These animals were compared

with a similarly exposed group that was untreated (n=10). Reactivity was determined by measuring specific airway resistance (SRaw) upon intravenous acetylcholine infusion in unanesthetized, spontaneously breathing animals. Prior to ozone exposure, we found that drug treatment did not affect either SRaw or muscarinic reactivity. After exposure to 3.0 ppm, all untreated guinea pigs showed substantial muscarinic hyperreactivity. Indomethacin treatment did not inhibit this effect. Furthermore, in the indomethacin-treated animals, marked elevations occurred in SRaw after ozone. In contrast, no change in SRaw or muscarinic reactivity occurred after ozone in any animal treated with either BW 755C or FPL 55712. We conclude that ozone-induced bronchial hyperreactivity in the guinea pig rapidly develops after a brief, high-level exposure. This effect may be mediated in part by lipoxygenase products derived from lung arachidonic acid metabolism post-ozone.

Lee, H. K. and *Murlas, C.*

American Review of Respiratory Disease 132:1005-1009, 1985.

Other support: National Heart, Lung and Blood Institute.

From the Departments of Medicine and Environmental Health, University of Cincinnati School of Medicine, Cincinnati, OH.

U-60,257 INHIBITS 0_3-INDUCED BRONCHIAL HYPERREACTIVITY IN THE GUINEA PIG

We studied the effect on ozone-induced airway hyperreactivity of U-60,257, a pyrroloprostacyclin shown to inhibit leukotriene C/D biosynthesis in vitro. A group of 5 guinea pigs was pretreated with U-60,257 (5 mg/kg IV) and studied before and 30 min after a 15 min exposure to 3.0 ppm ozone. These animals were compared to a similarly exposed group that was untreated (n=10). Reactivity was determined by measuring specific airway resistance (SRaw) upon intravenous acetylcholine infusion in unanesthetized, spontaneously breathing animals. We found that U-60,257 treatment prior to ozone exposure did not affect either SRaw or muscarinic reactivity. After exposure to 3.0 ppm, all untreated guinea pigs showed substantial muscarinic hyperreactivity. In contrast, no significant change in SRaw or muscarinic reactivity occurred after ozone in any animal pretreated with U-60,257. We conclude that the ozone-induced bronchial hyperreactivity in the guinea pig rapidly develops after a brief, high level exposure. This effect may be mediated in part by leukotrienes generated upon ozone exposure.

Murlas, C. and Lee, H.K.

Prostaglandins 30(4):563-568, 1985.

Other support: National Heart, Lung and Blood Institute.

From the Departments of Medicine and Environmental Health, University of Cincinnati School of Medicine, Cincinnati, OH.

INDOMETHACIN INHIBITS THE AIRWAY HYPERRESPONSIVENESS BUT NOT THE NEUTROPHIL INFLUX INDUCED BY OZONE IN DOGS

To determine whether oxygenation products of arachidonic acid may be involved in the airway hyperresponsiveness induced by ozone exposure, we studied whether

ozone-induced hyperresponsiveness in dogs could be inhibited by the prostaglandin synthetase inhibitor, indomethacin. Airway responsiveness was assessed with dose-response curves of acetylcholine aerosol *versus* pulmonary resistance in 2 sets of experiments: in one set, 5 dogs were given no indomethacin treatment and were studied both before and after ozone exposure (3.0 ppm, 2 h); in another set, the same dogs were studied before indomethacin treatment or ozone exposure and then during treatment (1 mg/kg every 12 h for 4 days) both before and after ozone exposure. On each occasion, we also determined the number of neutrophils in biopsies of the airway epithelium. When the dogs were not treated with indomethacin, ozone caused a marked increase in responsiveness to acetylcholine and a marked increase in the number of neutrophils in the airway epithelium. When the dogs were given indomethacin, responsiveness was no different during treatment than before treatment, but more importantly, responsiveness did not increase significantly after they were exposed to ozone. Interestingly, indomethacin treatment did not affect either the baseline number of epithelial neutrophils before ozone exposure or the increase in the number of neutrophils after exposure. The results suggest that oxygenation products of arachidonic acid that are sensitive to inhibition by indomethacin play a role in ozone-induced hyperresponsiveness without affecting the influx of neutrophils.

O'Byrne, P. M., Walters, E. H., Aizawa, H., Fabbri, L. M., Holtzman, M. J., and *Nadel, J.*

American Review of Respiratory Disease 130:220-224, 1984.

Other support: National Heart, Lung and Blood Institute, Fisons Corporation and California Air Resources Board.

From the Cardiovascular Research Institute and Departments of Medicine and Physiology, University of California, San Francisco.

CONTROL OF NEUROTRANSMISSION BY PROSTAGLANDINS IN CANINE TRACHEALIS SMOOTH MUSCLE

Contractile responses of canine tracheal smooth muscle to electrical field stimulation diminished over a 2-h period of incubation. However, addition of indomethacin (10^{5}M) for a similar time not only prevented this inhibition of contractile response, but actually increased markedly the response to electrical field stimulation, suggesting that prostaglandins were responsible for the time-dependent inhibition. Measured prostaglandin E_2 increased in the tissue bath over 2-h in control tissues. Addition of prostaglandin E_2 to the tissue produced similar inhibition of contractile responses to electrical field stimulation in a concentration-dependent manner. In contrast, incubation alone, treatment with indomethacin or addition of prostaglandin E_2 had little, if any, effect on contractions induced by acetylcholine. We conclude that the release of prostaglandins from canine tracheal smooth muscle that occurs with time has a predominantly inhibitory effect on cholinergic neurotransmission at a prejunctional site.

Walters, E. H., O'Byrne, P. M., Fabbri, L. M., Graf, P. D., Holtzman, M. J. and *Nadel, J. A.*

Journal of Applied Physiology 57(1):129-134, 1984.

Other support: National Heart, Lung and Blood Institute, Fisons Corporation and California Air Resources Board.

From the Cardiovascular Research Institute and Departments of Medicine and Physiology, University of California, San Francisco.

AN ANTI-INFLAMMATORY DRUG (BW755C) INHIBITS AIRWAY HYPERRESPONSIVENESS INDUCED BY OZONE IN DOGS

To follow up our previous observation that airway hyperresponsiveness induced by ozone is linked to airway inflammation, we investigated the effect of BW755C, an anti-inflammatory drug, on ozone-induced hyperresponsiveness in dogs. Airway responsiveness was assessed with dose-response curves of acetylcholine aerosol *versus* pulmonary resistance in two sets of experiments. In one set (placebo treatment), five dogs were given only saline solution treatment and were studied before treatment or ozone exposure and then after treatment both before and after ozone (3.0 ppm, 2 hours); in another set (BW755C treatment), the same dogs were studied before BW755C treatment or ozone and then after treatment (10 mg/kg intravenously) both before and after ozone. When the dogs were not given BW755C, ozone induced a marked increase in airway responsiveness to acetylcholine. When the dogs were given BW755C, responsiveness was no different during treatment than before treatment but, more importantly, responsiveness did not increase significantly after ozone. It is concluded that BW755C markedly inhibits ozone-induced airway hyperresponsiveness in dogs, probably by inhibiting the formation of oxygenation products of arachidonic acid.

Fabbri, L. M., Aizawa, H., O'Byrne, P. M., Bethel, R. A., Walters, E. H., Holtzman, M. J., and *Nadel, J. A.*

Journal of Allergy and Clinical Immunology 76:162-166, 1985.

Other support: National Institutes of Health, Fisons Corporation, California Air Resources Board, and the Italian National Research Council.

From the Cardiovascular Research Institute and Departments of Medicine and Physiology, University of California, San Francisco.

PREDOMINANT GENERATION OF 15-LIPOXYGENASE METABOLITES OF ARACHIDONIC ACID BY EPITHELIAL CELLS FROM HUMAN TRACHEA

Epithelial cells of 99% purity and 92% viability were isolated from human tracheas obtained post mortem, and the cellular pathways for lipoxygenation of arachidonic acid were examined *in vitro*. The lipoxygenase metabolites were identified by comparison with synthetic standards during reversed-phase and straight-phase high pressure liquid chromatography, UV spectroscopy and gas chromatography/mass spectrometry. Epithelial cells incubated without arachidonic acid failed to generate detectable quantities of metabolites, while cells incubated with arachidonic acid at 1-50 μg/ml for 1-30 min invariably generated predominantly 15-lipoxygenase products, including 15-hydroxyicosatetraenoic acid (15-HETE), four isomers of 8,15-dihydroxyicosatetraenoic acid (two 8, 15 diHETES and two 8,15-leukotrienes), at least one isomer of 14,15-dihydroxyicosatetraenoic acid, and smaller amounts of 12-HETE and 8-HETE, but little or no detectable 5-HETE or 5,12-diHETEs. The capacity of epithelial cells from human pulmonary airway to selectively generate 15-lipoxygenase metabolites of arachidonic acid suggests a potential role for the products as mediators of airway epithelial function.

Hunter, J. A., Finkbeiner, W. E., *Nadel, J. A.*, Goetzl, E. J., and Holtzman, M. J.

Proceedings of the National Academy of Sciences of the United States of America 82:4633-4637, 1985.

Other support: National Institutes of Health and Cystic Fibrosis Research Foundation.

From the Cardiovascular Research Institute, Howard Hughes Medical Institute, and Departments of Medicine, Pathology and Physiology, University of California, San Francisco.

LEUKOTRIENE B_4 INDUCES AIRWAY HYPERRESPONSIVENESS IN DOGS

The authors studied the effect of leukotriene B_4 aerosols on airway responsiveness to inhaled acetylcholine aerosols and on the cellular components and cyclooxyngenase metabolites in bronchoalveolar lavage fluid in dogs. Inhalation of leukotriene B_4 aerosols had no effect on resting total pulmonary resistance but increased airway responsiveness, and effect that was maximum in 3 h and that returned to control levels within 1 wk. Three hours after leukotriene B_4, the number of neutrophils and the concentration of thromboxane B_2 recovered in lavage fluid increased markedly. Pretreatment with the thromboxane synthase inhibitor OKY-046 prevented the increases in airway responsiveness and in thromboxane B_2 but did not alter neutrophil chemotaxis. Thus we speculate that leukotriene B_4 causes neutrophil chemotaxis and release of thomboxane B_2, which increases airway responsiveness.

O'Byrne, P. M., Leikauf, G. D., Aizawa, H., Bethel, R. A., Ueki, I. F., Holtzman, M. J., and *Nadel, J. A.*

Journal of Applied Physiology 596(6):1941-1946, 1985.

Other support: National Heart, Lung and Blood Institute, Fisons Corporation, and the California Air Resources Board.

From the Cardiovascular Research Institute and Departments of Medicine and Physiology, University of California, San Francisco.

PROSTAGLANDIN $F_{2\alpha}$ INCREASES RESPONSIVENESS OF PULMONARY AIRWAYS IN DOGS.

The authors studied the effect of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) on the responsiveness of pulmonary airways in dogs. Airway responsiveness was assessed by determining the bronchoconstrictor response to increasing concentrations of acetylcholine aerosol delivered to the airways. In each of five dogs, we determined responsiveness during treatment with physiologic saline, histamine, or $PGF_{2\alpha}$ aerosols. The doses of histamine and $PGF_{2\alpha}$ were determined by establishing the largest dose of each which could be given to the dog without causing bronchoconstriction (subthreshold doses). We found that airway responsiveness was not significantly different during histamine treatment than after saline; however, responsiveness increased during treatment with $PGF_{2\alpha}$. In addition, the hyperresponsiveness induced by $PGF_{2\alpha}$ was prevented by pretreatment with the ganglion blocking drug hexamethonium (5 mg/kg given intravenously). The results show that $PGF_{2\alpha}$ specifically increases the responsiveness of pulmonary airways in doses that do not cause bronchoconstriction, and suggest that the hyperresponsiveness involves a neural mechanism such as increased responsiveness of airway sensory nerves.

O'Byrne, P. M., Aizawa, H., Bethel, R. A., Chung, K. F., *Nadel, J. A.*, and Holtzman, M.J.

Prostaglandins 28(4):537-543,1984.

Other support: National Heart, Lung and Blood Institute, the California Air Resources Board and Fisons Corporation.

From the Cardiovascular Research Institute and Departments of Medicine and Physiology, University of California, San Francisco.

NEUTROPHIL DEPLETION INHIBITS AIRWAY HYPERRESPONSIVENESS INDUCED BY OZONE EXPOSURE

The authors studied whether ozone-induced hyperresponsiveness could be inhibited by neutrophil depletion in dogs. Responsiveness was assessed with dose-response curves of acetylcholine aerosol *versus* pulmonary resistance; depletion was assessed by counting neutrophils in venous blood and in biopsies of the airway epithelium. Responsiveness and neutrophil numbers were determined 5 days and 1 day before ozone and 1 h after ozone (3.0 ppm, 2 h) in 6 untreated dogs and in 6 dogs treated with hydroxyurea (200 mg/kg daily for 5 days starting 5 days before ozone). In untreated dogs, responsiveness and neutrophil numbers 5 days and 1 day before ozone did not change, but responsiveness and epithelial neutrophils increased markedly after ozone. In treated dogs, circulating neutrophils decreased from 8.9 ± 2.2 to 0.6 ± 0.01 X 10^3 per mm^3 (mean ± SEM), and responsiveness before ozone did not change. Furthermore, increases in responsiveness and epithelial neutrophils did not occur after ozone. Six wk after stopping hydroxyurea, responsiveness and epithelial neutrophils increased markedly after ozone. The results suggest that ozone-induced hyperresponsiveness may depend on the mobilization of neutrophils into the airways.

O'Byrne, P. M., Walters, E. H., Gold, B. D., Aizawa, H. A., Fabri, L. M., Alpert, S. E., *Nadel, J. A.*, and Holtzman, M. J.

American Review of Respiratory Disease 130:214-219, 1984.

Other support: National Heart, Lung and Blood Institute and Fisons Corporation.

From the Cardiovascular Research Institute, and Departments of Medicine and Physiology, University of California, San Francisco.

AIRWAY HYPERRESPONSIVENESS AND CHANGES IN CELL COUNTS IN BRONCHOALVEOLAR LAVAGE AFTER OZONE EXPOSURE IN DOGS

The authors studied whether airway hyperresponsiveness induced by ozone exposure is associated with changes in the numbers of different types of cells in bronchoalveolar lavage in dogs. Airway responsiveness to acetylcholine and the numbers of cells in lavage fluid were determined 1 wk before and then 1 h and 1 wk after 2-h exposures to filtered air and to ozone (3.0 ppm) in each of 5 dogs. Airway responsiveness and the numbers of cells in lavage fluid did not change after exposure to filtered air. By contrast, airway responsiveness increased markedly 1 h after exposure to ozone and returned to control levels 1 wk later. In addition, the numbers of neutrophils and of ciliated epithelial cells in lavage increased markedly 1 h after ozone and returned to control levels 1 wk later. Our previous study showed that airway hyperresponsiveness

induced by ozone is associated with an influx of neutrophils into the most central airways; the present results suggest that the hyperresponsiveness is also accompanied by an influx of neutrophils into more distall airways and by desquamation of airway epithelial cells.

Fabri, L. M., *Nadel, J. A., et al.*

American Review of Respiratory Disease 129:288-291, 1984.

Other support: National Heart, Lung and Blood Institute, Fisons Corporation and the California Air Resources Board.

From the Cardiovascular Research Institute, Departments of Medicine and Physiology, University of California, San Francisco.

OCCURRENCE AND DISTRIBUTION OF REGULATORY PEPTIDES IN THE RESPIRATORY TRACT

A multimembered system of active regulatory peptides has been found in the respiratory tract. Studies on the neuroendocrine marker, neuron-specific enolase, indicate that there are still further types of mucosal endocrine cells and nerves whose products remain to be elucidated. This is not surprising in view of the rate of discovery of novel regulatory peptides by new biochemical methods and recombinant DNA technology. It is well known that the lung possesses functions in addition to mere respiration and these functions may well be regulated by the various regulatory peptides present in the respiratory tract. Many diseases of the lung are still poorly understood. The discovery of regulatory peptides involved in regulating mucosecretion, blood flow and muscle tone will undoubtedly contribute to the further understanding of these diseases.

The basic mechanism of tumor growth and the biology of tumor cells will also be better understood after further study of the active regulatory peptides, many of which play an important role as autocoids in the maintenance of tumor growth.

Polak, J. M. and Bloom, S. R.

Cancer Research 99:1-16, 1985.

From the Departments of Histochemistry and Medicine, Royal Postgraduate Medical School, Hammersmith Hospital, London, England.

BRONCHODILATOR RESPONSE DURING ACUTE VIRAL BRONCHIOLITIS IN INFANCY

Bronchodilator responsiveness was assessed by measuring specific respiratory conductance before and after inhalation of aerosolized bronchodilator in 50 infants who had acute bronchiolitis due to respiratory syncytial virus infection. Thirty percent of the infants showed an improvement in specific conductance. Responders could not be differentiated from nonresponders by family histories of atopy, eosinophil counts or immunoglobulin levels in blood and nasal secretions. Eighty-three percent of the families and 54% of the mothers of the infants were smokers. Babies of smoking mothers had lower specific conductances than did those of nonsmoking mothers but showed no differences in bronchodilator response. The clinical significance of this bronchodilator-responsive subgroup has yet to be defined.

Soto, M. E., Sly, P., Uren, E., *Taussig, L. M.*, and Landau, L.

Pediatric Pulmonology 1(2):85-86, 1985.

Other support: National Institutes of Health.

From the Professional Department of Thoracic Medicine, Royal Children's Hospital, Melbourne, Australia.

UTILIZATION OF A PEROXIDASE ANTIPEROXIDASE COMPLEX IN AN ENZYME-LINKED IMMUNOSORBENT ASSAY OF ELASTIN-DERIVED PEPTIDES IN HUMAN PLASMA

Chronic obstructive pulmonary disease (COPD), a major cause of morbidity and death in the smoking population, develops insidiously over many years, and significant impairment of lung function usually occurs before the disease is diagnosed. Because lung elastin degradation appears to be a prerequisite for the development of the disease, immunologic detection of elastin-derived peptides in the blood might be an effective approach to the early detection and monitoring of the disease. We here report an improved enzyme-linked immunosorbent assay for elastin peptides using a peroxidase-antiperoxidase complex as the reporter group. The assay is sensitive to 2ng/ml elastin peptides. We show that for optimal, reproducible results the assay should be carried out at 16° C rather than at room temperature and that determinations should be made on plasma containing protease inhibitors rather than on serum. The levels of elastin-derived peptides appeared to remain relatively constant when multiple samples were taken during a 5- to 10-wk period from individual subjects. In addition, patients with COPD had elevated elastin peptide levels (127±47ng/ml), whereas normal smokers had values intermediate between the 2 groups (mean peptide levels of 76±42ng/ml) compared with levels in normal nonsmokers (58±17ng/ml). A small group of normal smokers (20%) had elevated peptide levels similar to those in the emphysema group and may represent that groups of smokers at risk of developing obstructive lung disease.

Kucich, U., *Weinbaum, G., et al.*

American Review of Respiratory Disease 131:700-713, 1985.

Other support: National Institutes of Health.

From the Department of Medicine, Graduate Hospital, Albert Einstein Medical Center and Center for Oral Health Research, School of Dental Medicine, University of Pennsylvania, Philadelphia.

AMINO ACID DERIVED LATENT ISOCYANATES: IRREVERSIBLE INACTIVATION OF PORCINE PANCREATIC ELASTASE AND HUMAN LEUKOCYTE ELASTASE

Several amino acid derived azolides have been synthesized and investigated for their inhibitory activity toward human leukocyte elastase and porcine pancreatic elastase. The inhibitory activity was found to be dependent on the nature of the precursor amino acid ester. Thus, compounds derived from L-valine methyl ester 3, L-norvaline methyl ester 5, DL-norleucine methyl ester 9, and L-methionine methyl ester 10, were found to inhibit irreversibly both enzymes. Compound 10 was found to be a specific and selective inhibitor of human leukocyte elastase. In contrast to these, inhibitors derived from glycine methyl ester 1, D-valine methyl ester 4, and D-norvaline methyl ester 6 were

found to be inactive. The results of the present study show that latent isocyanates derived from appropriate amino acids can serve as selective inhibitors of serine proteases and are of potential pharmacological value.

Groutas, W. C., *Weinbaum, G., et al.*

Journal of Medicinal Chemistry **28**(2):204-209, 1985.

Other support: American Chemical Society, American Lung Association and National Heart, Lung and Blood Institute.

From the Department of Chemistry, Wichita State University, Wichita, KS; the Graduate Hospital, Department of Medicine, Research Division, Philadelphia; the Department of Veterinary Biosciences, University of Illinois, Urbana; and the Department of Chemistry, University of Wisconsin, Eau Claire.

COLLAGENASE IN THE LOWER RESPIRATORY TRACT OF PATIENTS WITH ADULT RESPIRATORY DISTRESS SYNDROME

Collagenase activity in the bronchoalveolar lavage (BAL) of patients with adult respiratory distress syndrome (ARDS) was measured against Type I collagen (17 patients) and against Type III collagen (13 patients). Serine protease activity was also measured against Type III collagen (13 patients). Type I collagenase activity was detectable in 12 of 17 and Type III collagenase was detectable in 12 of 13 patients with ARDS. The 10 control subjects had no detectable Type I or III collagenase activity. Total and differential white cell counts were analyzed in the lavage fluid. Although the total counts did not differ between patients with ARDS and control subjects, the percentage of neutrophils was increased more than 25-fold and the percentage of macrophages was reduced almost 10-fold in the ARDS patients. Serial collagenase activity was followed in 1 ARDS survivor. In this patient Type III collagenase activity peaked before the Type I collagenase activity or serine protease activity reached their maximums. Both the last two enzyme activities paralleled the total recoverable cells in the BAL.

Christner, P., *Weinbaum, G., et al*

American Review of Respiratory Disease 131:690-695, 1985.

Other support: U. S. Public Health Service.

From the Division of Pulmonary and Critical Care Medicine, Albert Einstein Medical Center, New York; Temple University Health Science Center; the Department of Dental Histology, University of Pennsylvania; and the Division of Research, Department of Medicine, Graduate Hospital, Philadelphia.

SECRETION OF ELASTIN IN THE EMBRYONIC CHICK AORTA AS VISUALIZED BY IMMUNOELECTRON MICROSCOPY

Recently, significant advances have been made in characterizing the pathway of elastin biosynthesis from the biochemical point of view and a 70,000 dalton protein, designated tropoelastin, appears to be the primary translation product and soluble intermediate of the insoluble elastin. However, relatively little is known concerning the intracellular secretory pathway of tropoelastin. We previously developed an electron microscopic technique using elastin-specific antibody and ferritin-conjugated secondary

antibody to identify intracellular elastin and to identify, provisionally, intracellular vesicles containing elastin. However, the method did not permit localization of elastin in other intracellular organelles. We now describe an improved post-embedding technique using the peroxidase-antiperoxidase method to detect the primary elastin antibody; we have localized elastin in both the endothelial and medial cells of the embryonic chick aorta. Specific staining was visualized in the cisternae of the endoplasmic reticulum, in the Golgi apparatus, and in vesicles forming on the trans side of the Golgi. Some of these smaller vesicles appeared to fuse, forming larger vesicles which may have a storage function. Both types of vesicles were seen fusing with the cell plasma membrane, suggesting that elastin is secreted by an exocytotic process. These results suggest that tropoelastin follows the classical pathway for protein secretion.

Damiano, V., Tsang, A. L., *Weinbaum, G.*, Christner, P., and Rosenbloom, J.

Collagen Related Research 4:153-164, 1984.

Other support: National Institutes of Health.

From the Center for Oral Health Research, School of Dental Medicine; the Department of Medicine, Graduate Hospital; and University of Pennsylvania, Philadelphia.

PROTEINASE INHIBITORY FUNCTION IN INFLAMMATORY LUNG DISEASE I. ACUTE BACTERIAL PNEUMONIA

This study examines bronchial alveolar lavage (BAL) samples from a group of patients with acute bacterial pneumonia (n=13) and makes a comparison with a control group (N=5). The proteinase inhibitory capacity was examined and found to be composed primarily of alpha$_1$-proteinase inhibitor (PI; alpha$_1$-antitrypsin) and, to a lesser extent, bronchial mucosal inhibitor. Although the average PI concentration was elevated approximately 5-fold in the pneumonia group, its inhibitory function against elastase was decreased 15-fold when compared with that in the control group. The pneumonia group showed an increased concentration of immunologically identified elastin-derived peptides. Some of the BAL fluid from patients with pneumonia showed elastolytic activity against amorphous insoluble lung elastin. The majority of the elastase appears to be of neutrophil origin. Bronchial mucosal inhibitor is shown to be a component of both normal and pneumonia BAL fluids by both immunologic quantitation and by its resistance to perchloric acid inactivation. Compared with those from control subjects, BAL samples from patients with acute bacterial pneumonia showed a decreased proteinase inhibitor function and both increased elastolytic activity and elastin-derived peptide concentration.

Abrams, W. R., *Weinbaum, G. et al.*

...rican Review of Respiratory Disease 129:735-741, 1984.

Other support: National Heart, Lung and Blood Institute.

From the Graduate Hospital, Department of Medicine, Research Division; Albert Einstein Medical Center, Department of Medicine, Pulmonary and Critical Care Divisions; Temple University Health Science Center, Pulmonary Disease Section, Philadelphia, and the Pulmonary Disease Department, Saga Medical School, Japan.

NEUTROPHIL ELASTASE-RELEASING FACTORS IN BRONCHOALVEOLAR LAVAGE FROM PATIENTS WITH ADULT RESPIRATORY DISTRESS SYNDROME

Bronchoalveolar lavage fluid (BAL) was obtained from patients with adult respiratory distress syndrome (ARDS). Controls included BAL from normal subjects and from patients with sarcoidosis or pulmonary fibrosis. Neutrophil elastase measured immunologically was found in all BAL samples, but it was strikingly greater in BAL from patients with ARDS than in the BAL from normal subjects or patients with sarcoidosis. There was no significant difference in the neutrophil elastase antigen concentrations in BAL samples from patients with ARDS and those with pulmonary fibrosis. No elastolytic activity was found in either group. The alpha-1-antitrypsin and the bronchial mucus inhibitor were greater in BAL from patients with ARDS. There was a highly significant correlation between the alveolar-arterial oxygen tension difference and the neutrophil elastase concentration in BAL from the patients with ARDS. Kallikrein, prerekallikrein, factor XIa-like activity, and high molecular weight kininogen antigen were found in BAL of patients with ARDS, suggesting that the kallikrein-kinin cascade may be activated in the lungs of patients with ARDS. Kallikrein-like activity in the BAL from the patients with ARDS was significantly correlated with the number of neutrophils in the BAL, the neutrophil elastase concentration, and the ability of the BAL to release elastase from cytochalasin-B-treated neutrophils. There was no correlation between these variables and C5a concentration. These studies demonstrated an association between BAL neutrophil elastase and the clinical state of patients with ARDS.

Idell, S., *Weinbaum, G. et al.*

American Review of Respiratory Disease 132:1098-1105, 1985.

Other support: National Heart, Lung and Blood Institute, National Institutes of Health and American Heart Association, Pennsylvania Affiliate.

From the Pulmonary Disease and Hematology-Oncology Sections, Department of Medicine, and Thrombosis Research Center, Temple University Medical School; Pulmonary Disease Section, Albert Einstein Medical Center, Northern Division, Philadelphia.

OXIDANTS INCREASE PARACELLULAR PERMEABILITY IN A CULTURED EPITHELIAL CELL LINE.

Inflammation of epithelia is an important step in the pathophysiology of a wide variety of diseases. Because reactive oxygen metabolites are important effector molecules of acute inflammation, we examined the effect of oxidants on the barrier function of a cultured epithelium, Madin Darby Canine Kidney cells, by measuring the transepithelial electrical conductance, G_t, of monolayers grown on permeable supports. We found that H_2O_2, added directly or generated with glucose oxidase, increased G_t. Similar effects were observed with addition of xanthine and xanthine oxidase, a system which enzymatically generates superoxide radical O_2^-. The oxidant-induced increase in conductance was reversible if the exposure to oxidants was not prolonged ($t \leq 20$ min), and if the concentration of H_2O_2 was $< 5 \times 10^{-3}$ M. The increase in G_t suggested that oxidants increase the permeability of the paracellular pathway, a suggestion supported by an oxidant-induced increase in the permeability to ^{14}C-mannitol, which primarily crosses epithelia via the extracellular route.

In addition to functional changes in the epithelial monolayer, oxidants changed the cell morphology; after H_2O_2 exposure, the cells tended to pull apart, most prominently at their basolateral surfaces. These changes were heterogeneous, with most areas showing no changes. Some of the morphologic changes could be reversed if the exposure to H_2O_2 was limited. We also observed a disruption of the normal pattern of the actin-cytoskeleton, particularly in the area of cell to cell junctions, as demonstrated by fluorescent staining of f-actin with rhodamine phallicidin.

These functional and structural findings indicate that oxidants increase the permeability of the paracellular pathway in a cultured epithelium. The changes can be reversible and are accompanied by alterations in organization of the cell cytoskeleton. These studies demonstrate the dynamic nature of the interaction between epithelial cells and oxygen metabolites.

Welsh, M. J., Shasby, D. M., and Husted, R. M.

Journal of Clinical Investigation 76:1155-1168, 1985.

Other support: National Institutes of Health, American Heart Association and Veterans Administration.

From the Laboratory of Epithelial Transport and Pulmonary Division, Department of Internal Medicine, University of Iowa College of Medicine, and Veterans Administration Hospital, Iowa City.

CHARACTERIZATION OF LIPOPROTEIN PARTICLES ISOLATED BY IMMUNOAFFINITY CHROMATOGRAPHY

Two populations of A-I-containing lipoprotein particles: A-I-containing lipoprotein with A-II (Lp (A-I with A-II)), and A-I containing lipoprotein without A-II (Lp (A-I without A-II)) have been isolated from plasma of 10 normolipidemic subjects by immunoaffinity chromatography and characterized. Both types of particles possess α-electrophoretic mobility and hydrated density in the range of plasma high-density lipoproteins (HDL). Lp (A-I without A-II) and Lp (A-I with A-II) are heterogeneous in size. Lp (A-I without A-II) comprised two distinct particle sizes with mean apparent molecular weight and Stokes diameter of 3.01×10^5, and 10.8 nm for Lp (A-I without A-II)$_1$, and 1.64×10^5, and 8.5 nm for Lp (A-I without A-II)$_2$. Lp (A-I with A-II) usually contained particles at least three distinct molecular sizes with mean apparent molecular weight and Stokes diameter of 2.28×10^6 and 9.6 nm for Lp (A-I with A-II)$_1$, 1.80×10^5 and 8.9 nm for Lp (A-I with A-II)$_2$, and 1.25×10^5 and 8.0 nm for Lp (A-I with II)$_3$. Apoproteins C, D and E, and lecithin:cholesterol acyltransferase (LCAT) were detected in both Lp (A-I without A-II) and Lp (A-I with A-II) with most of the apoprotein D, and E, and LCAT (EC 2.3.1.43) in Lp (A-I with A-II) particles. Lp (A-I without A-II) had slightly higher lipid/protein ratio than Lp (A-I with A-II). Lp (A-I with A-II) had an A-I/A-II molar ratio of approximately 2:1. The percentage of plasma A-I associated with Lp (A-I without A-II) was highly correlated with the A-I/A-II ratio of plasma ($r = 0.96$, $n = 10$). The variation in A-I/A-II ratio of HDL density subfractions therefore reflects different proportions of two discrete types of particles: particles containing A-I and A-II in a nearly constant ratio and particles containing A-I but no A-II. Each type of particle is heterogeneous in size and in apoprotein composition.

Cheung, M. C. and *Albers, J. J.*

The Journal of Biological Chemistry 259(19):12201-12209, 1984.

Other support: National Institutes of Health and the American Cancer Society.

From the Department of Medicine, University of Washington, Seattle.

PLATELET-DERIVED GROWTH FACTOR AND MALIGNANT TRANSFORMATION

Studies on human platelet-derived growth factor (PDGF) described in this paper represent the collective effort of several teams of investigators over the past 10 years. These studies produced an abundance of information concerning the nature and structure of PDGF, its role in cell growth and its diverse functions affecting cell migration, metabolic processes and receptor modulation. This work also led to an important discovery linking this potent mitogen to the transforming protein of the simian sarcoma virus, providing a basis for the understanding of the processes involved in transformation induced by the SSV *onc* gene. Specifically, the findings described here provided the missing link for the understanding of the mechanism of cell transformation induced by the *onc* gene of the simian sarcoma virus. This *onc* gene, *v-sis*, was shown to encode a PDGF-like polypeptide which is a potent mitogen for fibroblasts, arterial smooth muscle

cells and glial cells. Activation of *sis* transcription may cause the sustained abnormal proliferation of cells responsive to the mitogenic effects of the PDGF-like molecule. Overall, these findings are consistent with the suggestion that *sis* activation might be involved in the process leading normal cells of certain types towards malignancy.

Antoniades, H.N.

Biochemical Pharmacology 3(18):2823-2828, 1984.

Other support: U.S. Public Health Service and the American Cancer Society.

From the Center for Blood Research and the Department of Nutrition, Harvard School of Public Health, Boston.

SYNTHESIS AND SECRETION OF PROTEINS RESEMBLING PLATELET-DERIVED GROWTH FACTOR BY HUMAN GLIOBLASTOMA AND FIBROSARCOMA CELLS IN CULTURE

Immunoprecipitation of proteins extracted from metabolically labeled human glioblastoma and fibrosarcoma cells with antiserum to platelet-derived growth factor (PDGF) showed that these cells express and secrete proteins that are recognized specifically by the antiserum. The molecular masses of immunoprecipitated proteins in the lysates of the malignant cells ranged from 16 kDa to 140 kDa. Both cell lines secreted a 31-kDa polypeptide with structural, immunological and biological properties similar to those of human PDGF. These cell lines were shown to synthesize a 4.4-kb mRNA that contained sequences from all the six currently identified exons of the human c-*sis* gene. These data suggest that the PDGF-like proteins in the two mesenchyme-derived transformed cells are encoded at least in part by the c-*sis* locus.

In this report we describe the intracellular synthesis of PDGF-like proteins in cultures of human glioblastoma and fibrosarcoma cells and correlate the presence of these proteins with a c-*sis* mRNA species transcribed by the human c-*sis* locus. The intracellular PDGF-like proteins appear to be precursor and processed molecules of a major secreted protein of 31 kDa that is structurally, immunologically and functionally similar to human PDGF.

Pantazis, P., Pelicci, P.G., Dalla-Favera, R., and *Antoniades, H.N.*

Proceedings of the National Academy of Sciences of the United States of America 82:2404-2408, 1985.

Other support: National Institutes of Health and the American Cancer Society.

From the Center for Blood Research and Department of Nutrition, Harvard School of Public Health, Boston, and the Department of Pathology, Kaplan [illegible] Center, New York University School of Medicine, New York.

PLATELET-DERIVED GROWTH FACTOR POLYPEPTIDES IN HUMAN MEGAKARYOBLASTIC-LIKE CELL LINES

Novel cell lines derived from human peripheral blood were shown to synthesize polypeptides recognized by specific antisera to human platelet-derived growth factor (PDGF). Metabolically labeled intracellular polypeptides were immunoprecipitated by PDGF antiserum and were analyzed by SDS-polyacrylamide gel electrophoresis. The

molecular masses of the intracellular forms of PDGF-like polypeptides in these cell lines ranged from 12,000 to 48,000 daltons (12-48 kD), suggesting the presence of PDGF precursor and processed molecules. Metabolically labeled polypeptides immunoprecipitated by PDGF antisera were also recognized in the conditioned media of one of the cell lines obtained from a leukemic patient. The molecular masses of the secreted PDGF-like polypeptides were 12 kD, 31 kD, and 46 kD. Upon reduction, the 31-kD polypeptide was reduced to 16 kD, suggesting that it represents a disulfide-linked dimer similar to that described for PDGF.

Pantazis, P., Morgan, D.A., Brodsky, I., and *Antoniades, H.N.*

In: *CANCER CELLS 3/Growth Factors and Transformation*: Cold Spring Harbor Laboratory, 1985, pp. 153-157.

Other support: National Institutes of Health and American Cancer Society.

From the Center for Blood Research and Department of Nutrition, Harvard School of Public Health, Boston, and the Department of Hematology/Oncology, Hahnemann University, Philadelphia.

EFFECT OF CALCIUM ON THE STABILITY OF THE PLATELET MEMBRANE GLYCOPROTEIN IIb-IIIa COMPLEX

Platelet membrane glycoproteins IIb and IIIa form a Ca^{2+}-dependent heterodimer complex that contains binding sites for fibrinogen, von Willebrand factor, and fibronectin following platelet stimulation. The authors have studied the effect of Ca^{2+} on the stability of the IIb-IIIa complex using a IIb-IIIa complex specific monoclonal antibody A_2A_9 to detect the presence of the complexes. Soluble IIb and IIIa interacted with A_2A_9-Sepharose only in the presence of Ca^{2+} with 50% IIb-IIIa binding requiring 0.4 μM Ca^{2+}. In contrast, at 25°C the binding of radioiodine labelled A_2A_9 to intact unstimulated platelets suspended in buffers containing EDTA or ethylene glycol bis(β-aminoethyl ether) *N,N,N',N'*-tetracetic acid was independent of the presence of Ca^{2+}. However, the effect of Ca^{2+} chelators on ^{125}I-A_2A_9 binding varied with temperature. At 37°C, ^{125}I-A_2A_9 binding to intact platelets became Ca^{2+}-dependent with 50% binding requiring 0.4 μM Ca^{2+}. This effect of temperature was not due to a change in platelet membrane fluidity because enrichment or depletion of platelet membrane cholesterol did not influence antibody binding. Also, ^{125}I-A_2A_9 binding to intact platelets at 25° C became Ca^{2+}-dependent when the pH was increased above 7.4. At 1 nM Ca^{2+} and 25°C, 50% antibody binding occurred at pH 9.0. These studies demonstrate that Ca^{2+}- dependent IIb-IIIa complexes are present on unstimulated platelets and that the Ca^{2+} binding sites responsible for the stability of these complexes are located on the external platelet surface. These experiments also suggest that changes in platelet cytosolic Ca^{2+} do not regulate the formation of IIb-IIIa complexes.

Brass, L. F., Shattil, S. J., Kunick, T. J., and *Bennett, J. S.*

The Journal of Biological Chemistry 260(13):7875-7881, 1985.

Other support: National Institutes of Health and the American Heart Association.

From the Hematology-Oncology Section and the Department of Pathology and Laboratory Medicine, University of Pennsylvania School of Medicine, Philadelphia, and the Blood Center of Southeastern Wisconsin, Milwaukee.

THE TETRAPEPTIDE ANALOGUE OF THE CELL ATTACHMENT SITE OF FIBRONECTIN INHIBITS PLATELET AGGREGATION AND FIBRINOGEN BINDING TO ACTIVATED PLATELETS

Fibrinogen binding to receptors on activated platelets is a prerequisite for platelet aggregation. However, the regions of fibrinogen interacting with these receptors have not been completely characterized. Fibronectin also binds to platelet fibrinogen receptors. Moreover, the amino acid sequence Arg-Gly-Asp-Ser, corresponding to the cell attachment site of fibronectin, is located near the carboxyl-terminal region of the α-chain of fibrinogen. The researchers have examined the ability of this tetrapeptide to inhibit platelet aggregation and fibrinogen binding to activated platelets. Arg-Gly-Asp-Ser, but not the peptide Arg-Gly-Tyr-Ser-Leu-Gly, inhibited platelet aggregation stimulated by ADP, collagen, and γ-thrombin without inhibiting platelet shape change or secretion. At a concentration of 60-80 μM, Arg-Gly-Asp-Ser inhibited the aggregation of ADP-stimulated gel-filtered platelets ≈ 50%. Arg- Gly-Asp-Ser, but not Arg-Gly-Tyr-Ser-Leu-Gly, also inhibited fibrinogen binding to ADP-stimulated platelets. This inhibition was competitive with a K_i of about 25 μM but was incomplete even at higher tetrapeptide concentrations, indicating that Arg-Gly-Asp-Ser is a partial competitive inhibitor of fibrinogen binding. These data suggest that a region near the carboxyl-terminus of the α-chain of fibrinogen interacts with the fibrinogen receptor on activated platelets. The data also support the concept that the sequence Arg-Gly-Asp-Ser has been conserved for use in a variety of cellular adhesive processes.

Gartner, T. K. and *Bennett, J. S.*

The Journal of Biological Chemistry 260(22):11891-11894, 1985.

Other support: National Institutes of Health.

From the Department of Biology, Memphis State University, Memphis, and the Hematology-Oncology Section and Cancer Center, University of Pennsylvania School of Medicine, Philadelphia.

CARDIAC INOTROPIC RESPONSE OF A NEW β-1-AGONIST (TA-064) WITH LOW SARCOLEMMAL ADENYLATE CYCLASE ACTIVATION

These experiments were concerned with an investigation of the effect of a new selective β-1-adrenergic compound, TA-064, on the function of purified sarcolemmal vesicles prepared from perfused canine heart preparations. Special emphasis was placed on the effect of TA-064 on adenylate cyclase activity, on ATP-dependent Ca^{++} uptake and on phosphorylation of purified sarcolemma (SL). Perfusion was carried out in a supported canine heart preparation with a perfluorochemical (FC-43) as perfusate. TA-064 (317 μg) was injected into the left atrium. As previously reported, this resulted in a marked positive inotropic and chronotropic effect which was abolished by propranolol. Purity of the SL preparation was confirmed with marker enzymes for mitochondria, sarcoplasmic reticulum and SL. In addition, Na^+Ca^{++}-exchange characteristic for SL was determined. Sidedness of the vesicles was ascertained by means

of adenylate cyclase activity using SL preparations with and without alamethicin. TA-064, as compared to l-isoproterenol, exerted little effect on adenylate cyclase activity. The addition of TA-064 either to the perfused whole heart preparation or to prepared SL vesicles resulted in small elevations of adenylate cyclase activity relative to l-isoproterenol. TA-064 increased ATP-dependent Ca^{++} uptake and phosphorylation. The relative minor response of adenylate cyclase to TA-064 was apparently sufficient to elevate both myocardial contractility and phosphorylation and Ca^{++} dependent ATPase activity. Therefore, TA-064 belongs together with prenalterol in a group of β-1-agonists with full inotropic activity but relatively diminished capacity to activate adenylate cyclase. The pharmacologic and clinical implications of these findings were discussed.

Bing, R. J. et al.

Current Therapeutic Research 36(6):1127-1144, 1984.

Other support: Hoover Foundation and the Charles A. Lindbergh Fund.

From the Huntington Medical Research Institutes, Huntington Memorial Hospital, Pasadena, CA.

CARDIAC PERFUSION, PAST AND PRESENT

This paper was presented at a symposium which was devoted in part to the role of lipids in relation to cardiac metabolism and function. The work of pioneers in this area, including Tigerstedt, Ludwig, Lamgendorf, Wild, and Ringer, is noted. These early results were dwelt upon, according to the author, because they determined the future course of cardiac metabolism. Determination of the nutrition of the human heart in situ was a direct and logical extension of their work. More recently the course of cardiac metabolism has been influenced by biochemistry and biophysics by separation of subcellular particles and organelles. Renewed stimulation for the study of the isolated mammalian heart has come from the laboratory of Neely *et al*, whose perfusion technique has been particularly useful in the understanding of the regulation of glycolysis in the ischemic and anoxic myocardium. Neely's perfusion studies were primarily carried out on the rat heart. This has limited the options for the study of glycolytic intermediates and glycolytic flux, excluding determinations of high energy phosphates, which necessitate specimens of greater weight. The use of perfluorochemicals has now made it possible to perfuse larger mammalian hearts using Neely's working heart preparation. The remainder of this discussion is concerned with hemodynamic and metabolic data on the different types of the failing rabbit heart perfused *in vitro* with Fluosol-43. The effect of TA-064, a new positive inotropic agent, is briefly mentioned. In addition, this paper deals with a new approach to the problem of coronary spasm, using the isolated heart perfused with Fluosol-43 oxygenated with a bubble oxygenator.

Bing, R. J.

In: Ferrari, R., Katz, A., Shug, A., and Visioli, O. (eds.): *Myocardial Ischemia and Lipid Metabolism*, New York: Plenum Publishing Corporation, 1984, pp. 267-281.

Other support: Hoover Foundation.

From the Huntington Medical Research Institutes and Huntington Memorial Hospital, Pasadena, CA.

EFFECT OF INTACT ENDOTHELIUM AGAINST PLATELET-INDUCED CORONARY ARTERY SPASM IN ISOLATED RABBIT HEARTS

Effects of collagen-activated washed rabbit platelets on coronary arteries with and without intact endothelium were studied in a supported rabbit heart preparation using a perfluorocarbon (FC-43) as perfusate. The vascular diameter of obtuse marginal coronary arteries was determined by means of gated color arteriography (injection of patent blue dye). Endothelial denudation of the obtuse marginal artery was accomplished by scraping the lumen with a roughened plastic tubing. Injection of washed platelets (10 ml with about 500,000 platelets/μl) not treated with collagen failed to constrict coronary arteries either with intact or denuded endothelium. In contrast, injection of platelet suspension immediately after activation with collagen caused vasoconstriction of denuded obtuse marginal coronary arteries in 10 of 14 cases. In 6 preparations, occlusion was complete, lasting up to 16 minutes. In arteries with intact endothelium, no coronary vasoconstriction occurred. In hearts with coronary artery spasm, total coronary vascular resistance increased significantly. This study furnishes additional evidence that endothelial lesions are a contributory factor for large coronary artery spasm and that endothelial cells possess a protective function against vasoconstrictor substances released from aggregating platelets.

Bing, R. J., et al.

American Journal of Cardiology 55:1596-1600, 1985

Other support: Hoover Foundation and the Charles A. Lindbergh Fund.

From the Huntington Medical Research Institutes, Huntington Memorial Hospital, Pasadena, CA.

THE EFFECT OF NIMODIPINE, A CALCIUM ANTAGONIST, ON INTRACORTICAL ARTERIOLES IN THE CAT BRAIN

The purpose of this study was to test the effect of the calcium antagonist nimodipinine on the intracortical arterioles and on cerebral blood flow in the anesthetized cat. Arteriolar diameter, red cell velocity and regional cerebral blood flows were determined by a technique employing transillumination and high speed cinematography. Global cerebral blood flow was measured using the microsphere technique. After a five-minute infusion of nimodipine (0.625 μg/kg), there was a significant increase in cortical arteriolar diameter ($p < 0.001$) and in regional blood flow ($p < 0.01$). Red cell velocity increased ($p < 0.01$). Global cerebral blood flow increased ($p < 0.02$) while mean arterial blood pressure declined ($p < 0.05$). Therefore, direct observations confirm that nimodipine dilates intracortical arterioles and increases red cell velocity in these vessels.

Schmidli, J., Santillan, G. G., Saeed, M., Palmieri, D., and *Bing, R. J.*

Current Therapeutic Research 38(1):94-103, 1985.

Other support: Hoover Foundation and the Charles A. Lindbergh Fund.

From the Huntington Medical Research Institutes, Huntington Memorial Hospital, Pasadena, CA.

BETA-1-ADRENOCEPTOR AGONISTS WITH LOW ADENYLATE CYCLASE ACTIVATION — THEORETICAL AND CLINICAL IMPLICATIONS

Several partial beta-agonists such as TA-064, prenalterol, ICI89,963, and ICI119,033 possess pronounced positive inotropic effects, but induce only limited activation of myocardial adenylate cyclase; this results in low cyclic AMP levels. Partial agonists, as compared to isoproterenol, need high receptor occupancy for maximal physiological response and demonstrate inadequate coupling between receptor and adenylate cyclase. However, the biochemical mechanisms which generate the final biological effects originating from cyclic AMP are tissue-specific, and for that reason, can maintain a pronounced positive inotropic action. Clinical data with TA-064 and prenalterol have established positive inotropic effects without significant changes in heart rate. Partial beta-agonists with diminished cyclic AMP may be less arrhythmogenic than full beta-1-agonists. The clinical importance of partial beta-1-agonists remains to be established, particularly with relevance to long-term exposure and inherent beta blockade.

Chemnitius, J. M. and *Bing, R. J.*

Canadian Journal of Cardiology 1(3):186-190, 1985.

Other support: Hoover Foundation and the Charles A. Lindbergh Fund.

From the Huntington Medical Research Institutes, Huntington Memorial Hospital, Pasadena, CA.

CRYSTALLOID AND PERFLUOROCHEMICAL PERFUSATES IN AN ISOLATED WORKING RABBIT HEART PREPARATION

Krebs-Henseleit buffer (KH) and a perfluorochemical (FC-43) were compared as perfusates in an isolated working rabbit heart preparation. Both perfusates were oxygenated in an identical manner using an infant bubble oxygenator. After 60 min of perfusion, no difference could be detected in the ratio of wet to dry heart weight between KH- and FC-43-perfused hearts (KH, 6.25 ± 0.3; FC-43, 5.99 ± 0.20). Left ventricular systolic pressure, maximal rate of left ventricular pressure rise, mean aortic systolic pressure, cardiac output, aortic flow, left ventricular power, and myocardial O_2 consumption (MVo_2) were significantly higher in FC-43-perfused hearts throughout the time of perfusion. However, there were no differences in resistance to cardiac output and heart rate. In KH- and FC-43-perfused hearts, MVo_2 and left ventricular power were closely correlated (KH, $r = 0.793$; FC-43, $r = 0.831$). Significantly higher coronary flow of KH-perfused hearts could be attributed to the lower viscosity of KH (1.05 Pa-s) compared with FC-43 (1.91 Pa-s) compared with FC-43 (1.91 Pa-s). Increased O_2 extraction during KH perfusion could not compensate for low O_2-carrying capacity of KH buffer (345 compared with 705 nmol O_2 ml^{-1} in FC-43 emulsion). A postischemic increase of coronary flow was observed only in FC-43-perfused hearts (28%). These results demonstrate a different response of perfused heart preparations to FC-43 and KH buffer.

Chemnitius, J. M., Burger, W., and *Bing, R. J.*

American Journal of Physiology 249(Heart Circ. Physiol.):H285-H292, 1985.

Other support: Hoover Foundation and the Charles A. Lindbergh Fund.

From the Huntington Medical Research Institutes, Huntington Memorial Hospital, Pasadena, CA.

A NEW METHOD FOR THE VISUALIZATION OF SUBEPICARDIAL CORONARY ARTERIES IN SMALL ISOLATED MAMMALIAN HEARTS

A model is described which permits direct visualization of large coronary arteries in a supported modified perfused heart preparation, using a perfluorochemical (FC-43) as perfusate. Filling of a large coronary artery with Patent Blue Dye is recorded by gated photography (color arteriography). The technique is applicable to the study of reactivity (spasm) of coronary arteries in hearts of small and large animals (rats, rabbits, dogs). The technique has the following advantages: preservation of vascular endothelium, adequate oxygenation, avoidance of major surgical intervention to implant sensors for the detection of changes in coronary diameter, quantitative evaluation of time-dependent changes in geometry of large coronary arteries, and simultaneous measurements of large coronary vessel and total coronary vascular resistance.

Burger, W., Chemnitius, J. M., and *Bing, R. J.*

Proceedings of the Society for Experimental Biology and Medicine 178:309-312, 1985

Other support: Hoover Foundation and the Charles A. Lindbergh Fund.

From the Huntington Medical Research Institutes, Huntington Memorial Hospital, Pasadena, CA.

THE EFFECT OF CARDIOPULMONARY BYPASS ON CEREBRAL BLOOD FLOW

These experiments deal with the effect of total cardiopulmonary bypass on global and regional cerebral blood flow. Global cerebral blood flow was determined with radioactive microspheres, regional cerebral flow by direct observation through transillumination and high-speed cinematography. The latter permitted observations of changes in caliber of cerebral arterioles and of red cell velocity. Arterial blood gas tension and pH were continously monitored. Measurements were carried out prior to and immediately following cardiopulmonary bypass. Studies on global cerebral flows showed that during cardiopulmonary bypass, cerebral blood flow rose and cerebral vascular resistance declined while blood pressure did not change significantly. During bypass, cerebral blood flow and blood pressure in animals treated as a group became interdependent and showed direct proportionality. Direct observations of the cortical microvasculature by means of high-speed cinematography revealed dilatation of cerebral cortical arterioles. Even though blood pressure remained unchanged, the diameter of cortical arterioles increased. Volume flow calculated from red cell velocity and arteriolar diameter showed a tendency of arteriolar flow to increase. The condition is reminiscent of the "luxury perfusion symdrome" (overabundant cerebral flow relative to metabolic needs of cerebral tissue).

Santillan, G. C., Chemnitius, J. M., and *Bing, R. J.*

Brain Research 345:1-9, 1985.

Other support: Hoover Foundation and the Charles A. Lindbergh Fund.

From the Huntington Medical Research Institutes, Huntington Memorial Hospital, Pasadena, CA.

A MODEL FOR THE STUDY OF CORONARY SPASM INDUCED CHANGES IN CARDIAC METABOLISM

A model is described which permits the study of localized and generalized arterial spasm in the intact working perfused rabbit heart with a perfluorochemical (FC-43) as perfusate. Coronary arteries were visualized by intraatrial injection of Patent Blue Dye with gated photography. Localized spasm resulted from topical spray of histamine (40 μmols) on the epicardial surface overlying an obtuse marginal artery. Before and following topical administration of histamine, regional coronary flow was determined using radioisotope-labeled microspheres. Generalized arterial spasm was initiated by intraatrial injection of histamine (10 μmols). After *topical administration*, obtuse marginal artery diameter decreased by 57%; large vessel resistance rose 32-fold; 20% rise of total coronary resistance resulted in a slight reduction of total coronary flow (16%). Heart rate, cardiac output, dP/dt_{max} and myocardial oxygen consumption did not change. However, regional coronary flow in the myocardium supplied by the affected artery diminished 21%, resulting in ischemic changes in redox pairs. After *intraatrial injection* of histamine, changes were more pronounced. Obtuse marginal artery diameter declined by 88%, resulting in 3300-fold rise of large vessel resistance. Total coronary resistance increased 150%, coronary flow and cardiac output diminished (56% and 24%). Both heart rate and dP/dt_{max} increased (16% and 17%). Generalized coronary spasm after intraatrial histamine injection resulted in several metabolic effects: Myocardial oxygen consumption (-48%); ATP (-29%); creatine phosphate (-34%); redox ratios, α-glycerophosphate/dihydroxyacetone phosphate and lactate/pyruvate, increased by 449% and 114%, respectively. The findings illustrate that localized and general coronary spasms can be produced and quantitated in a working heart model.

Burger, W., Chemnitius, J. M., Metz, M. Z., and *Bing, R. J.*

Journal of Molecular Cell Cardiology 17:917-930, 1985.

Other support: Hoover Foundation.

From the Huntington Medical Research Institutes, Huntington Memorial Hospital, Pasadena, CA.

THE EFFECT OF ISCHEMIA AND REPERFUSION ON SARCOLEMMAL FUNCTION IN PERFUSED CANINE HEARTS

This report deals with the effect of ischemia and reperfusion on purified sarcolemma obtained from canine myocardium of perfused heart preparation. Perfusion was carried out with a perfluorochemical (FC-43). Ischemia was produced by intermittent total clamping of inflow and outflow followed by reflow until the decrease in dp/dt_{max} had become stabile. Purity of sarcolemmal vesicles was ascertained with marker enzymes: succinate cytochrome c reductase (for mitochondria), K^+-stimulated p-nitrophenylphosphate (K^+-pNPPase), (Na^+/K^+) ATPase and adenylate cyclase (for SL). In addition Na^+/Ca^{++}-exchange characteristics for SL were determined. Sidedness of vesicles was ascertained by means of adenylate cyclase activity using sarcolemmal preparations treated and untreated with alamethicin. Emphasis was placed on ATP-dependent Ca^{++} uptake, phosphorylation of sarcolemmal vesicles and yield of SL proteins. Ischemia and reperfusion resulted in a significant reduction in adenylate

cyclase activity. This decline was significant following ischemia and reperfusion. The yield of protein recovered from SL vesicles from ischemic-reperfused heart preparations was also significantly decreased. Both initial rate of ATP-dependent Ca^{++} uptake and maximal Ca^{++} uptake fell significantly following ischemia and reperfusion. The initial rate of phosphorylation also dropped significantly. These disturbances in SL Ca^{++} transport following ischemia and reperfusion are probably a part of the general deficit in Ca^{++} translocation.

Chemnitius, J. M., Sasaki, Y., Burger, W., and *Bing, R. J.*

Journal of Molecular and Cellular Cardiology 17(12):1139-1150, 1985.

Other support: Hoover Foundation and the Lindbergh Fund.

From the Huntington Medical Research Institutes, Huntington Memorial Hospital, Pasadena, CA.

CLEAVAGE OF HUMAN HIGH MOLECULAR WEIGHT KININOGEN BY FACTOR XIa *IN VITRO*

We have recently demonstrated that human high molecular weight kininogen (HMWK) is a pro-cofactor that is cleaved by kallikrein to yield a two-chain cofactor (HMWKa) and the nanopeptide bradykinin. This proteolysis enhances its association with an activating surface, an event necessary for expression of its cofactor activity. We now report that factor XIa is capable of hydrolyzing HMWK and releasing bradykinin in a purified system as well as cleaving and inactivating HMWK in a plasma environment during the contact-activation process. The profile of proteolysis differs from that produced by kallikrein and by factor XIIa in that the first cleavage by factor XIa yield 75- and 45-kDa polypeptides, whereas both factor XIIa and kallikrein initially produce 65- and 56-kDa species. Further proteolysis by all three enzymes eventually produces similar heavy chains (M_r = 65,000) and light chains (45,000). However, the amount of factor XIa generated in plasma during contact-activation further degrades the light chain of HMWK, eventually destroying its coagulant activity. Furthermore, in a purified system, enhancement of the degradation of HMWK coagulant activity by factor XIa was achieved when kallikrein was included in the incubation mixture, suggesting that the preferred substrate for factor XIa is the active form of HMWK (HMWKa) and not the pro-cofactor. These data suggest that factor XIa has the potential to act as a regulator of contact-activated coagulation by virtue of its ability to destroy the cofactor function of HMWK after its generation by either kallikrein, factor XIIa, or to a lesser extent, factor XIa itself.

Scott, C. F., Silver, L. D., Purdon, A. D., and *Colman, R. W.*

The Journal of Biological Chemistry 260(19):10856-10863, 1985.

Other support: National Institutes of Health.

From the Thrombosis Research Center and the Hematology/Oncology Section of the Department of Medicine, Temple University Health Sciences Center, Philadelphia.

HUMAN PLASMA KALLIKREIN AND C1 INHIBITOR FORM A COMPLEX POSSESSING AN EPITOPE THAT IS NOT DETECTABLE ON THE PARENT MOLECULES: DEMONSTRATION USING A MONOCLONAL ANTIBODY

The inactivation of human plasma kallikrein (EC 3.4.21.8) by the inhibitor of activated complement component 1 (C1 inhibitor) induces the formation of a 1:1 stoichiometric kallikrein-C1 inhibitor complex and a proteolytically modified form of C1 inhibitor. We have produced a monoclonal antibody that recognizes the kallikrein-C1 inhibitor complex as well as modified C1 inhibitor, but fails to react with virgin C1 inhibitor or native plasma kallikrein. This observation constitutes an unequivocal demonstration that the reaction between plasma kallikrein and C1 inhibitor leads to the emergence of an epitope that is undetectable on the parent enzyme and inhibitor molecules.

Agostini, A. de, Schapiro, M., Wachtfogel, Y. T., *Colman, R.W.*, and Carrel, S.

Proceedings of the National Academy of Sciences of the United States of America 82:5190-5193, 1985.

Other support: Swiss National Science Foundation and U.S. National Institutes of Health.

From the Division de Rhumatologie, Hôpital Cantonal Universitaire, Geneva, Switzerland; Thrombosis Research Center and Hematology/Oncology Section, the Department of Medicine, Temple University School of Medicine, Philadelphia; and the Ludwig Institute for Cancer Research, Lausanne Branch, Epalinges, Switzerland.

PLASMA KALLIKREIN AND PRORENIN IN PATIENTS WITH HEREDITARY ANGIOEDEMA

Recent evidence indicates that plasma kallikrein is activated during acute attacks of hereditary angioedema. Plasma kallikrein is known to convert inactive renin, or prorenin, into an active proteolytic enzyme in plasma exposed to acid or low temperatures as well as in purified systems. To establish whether plasma kallikrein could activate prorenin under physiologic or pathologic conditions, prorenin to renin conversion was assessed at neutral pH in plasma deficient in C1 inhibitor (hereditary angioedema). In these plasma samples lacking the two major inhibitors of kallikrein and possessing ≤ 10% of the inhibitory activity of normal plasma, prorenin was not converted to an active enzyme despite conditions under which prekallikrein was completely activated to plasma kallikrein and despite normal prorenin concentrations and activability.

Purdon, A. D., Schapira, M., Agostini, A. de and *Colman, R. W.*

The Journal of Laboratory and Clinical Medicine 105(6):694-699, 1985.

Other support: National Institutes of Health and the Swiss National Science Foundation.

From the Thrombosis Research Center and Hematology/Oncology Section of the Department of Medicine, Temple University School of Medicine, Philadelphia, and the Division of Rhumatologie, Hôpital Cantonal Universitaire, Geneva, Switzerland.

PLATELET C1 INHIBITOR: A SECRETED ALPHA-GRANULE PROTEIN

In order to characterize which proteins of the contact phase of coagulation interact with platelets, human platelets were studied immunochemically and functionally to determine if they contain C1 inhibitor. By means of monospecific antibody to C1 inhibitor, a competitive enzyme-linked immunosorbent assay (CELISA) was developed to measure directly platelet C1 inhibitor. With the CELISA, from 33 to 115 ng of C1 inhibitor antigen per 10^8 platelets from 15 normal donors was quantified in lysates of washed human platelets solubilized in nonionic detergent. The mean concentration in 10^8 platelets was 62±33 ng (SD). Plasma C1 inhibitor either in the platelet suspension medium or on the surface of the platelets could account for only from 6.5 to 16% of the total antigen measured in the solubilized platelets. Upon functional studies, platelets contained 84±36 ng (SD) of C1 inhibitor activity in 10^8 platelets. As assessed by the CELISA, platelet C1 inhibitor antigen was immunochemically identical to plasma and purified C1 inhibitor. In contrast, the mean concentration of platelet C1 inhibitor antigen in platelets from four patients with classical hereditary angioedema was 8.3 ng/10^8 platelets (range 5.3 to 11.3 ng/10^8 platelets). 25 % and 31% of the totall platelet C1 inhibitor was secreted without cell lysis from normal platelets after exposure to collagen (20 μg/ml) and thrombin (1U/ml), respectively, and this secretion was blocked by metabolic inhibitors. Platelet subcellular fractionation showed that platelet C1 inhibitor resided mostly in alpha-granules, similar to the location of platelet fibrinogen. Thus, human platelets contained C1 inhibitor that became available by platelet secretion. The identification of platelet C1 inhibitor suggests that platelets may modulate the activation of the proteins of early blood coagulation and the classical complement pathways.

Schmaier, A. H., Smith, P. M. and *Colman, R. W.*

Journal of Clinical Investigation 75:242-250, 1985.

Other support: National Heart, Lung and Blood Institute; a Temple University Biomedical Research Support Grant; American Heart Association; and a Specialized Centers of Research grant.

From the Hematology/Oncology Section, Department of Medicine, Thrombosis Research Center, Temple University School of Medicine, Philadelphia.

EFFECT OF HEPARIN ON THE INACTIVATION RATE OF HUMAN ACTIVATED FACTOR XII BY ANTITHROMBIN III

Human antithrombin III (ATIII) is a plasma inhibitor of several serine proteases of the blood coagulation system. Previous investigations have reported that the presence of heparin has a multifold accelerating effect on the inhibition of factor XIIa and XIIf, the active species derived from factor XII. Recent studies from our laboratories have confirmed that ATIII inactivates factor XIIa and factor XIIf, but only contributes 2% to 3% to the inhibition of activated factor XII species in plasma. The major inhibitor is C1 inhibitor. Therefore, we have reexamined the heparin effect on the rate of inhibition of factor XIIa and factor XIIf in purified systems. We also have studied the effect of heparin on the inactivation of both factor XII-derived active species by various plasmas. Using purified factor XIIa and ATIII, we found that heparin (0.7 to 34.0 U/mL) increased the rate of inhibition of factor XIIa.

However, at heparin concentrations usually achieved during anticoagulant therapy (0.7 U/mL), the inhibition was accelerated only four-fold. This implies only a 6% contribution to the inhibitory effect of plasma. This suggestion was confirmed by the observation that heparin (1.5 U/mL) added to factor XIIa did not produce a detectable enhancement of the rate of inhibition of factor XIIa. Furthermore, using purified factor XIIf and antithrombin III, heparin (3.6 to 57.2 U/mL) increased the inactivation rate constant of factor XIIf by 1.6 to 14.0 times. This small effect was confirmed by the observation that heparin at a concentration greater than that sufficient for anticoagulation (1.4 U/mL) did not modify the inactivation rate of factor XIIf by prekallikrein-deficient plasma, and thus C1 inhibitor remains the major inhibitor even in the presence of heparin. From this study and our previous investigations on the effect of heparin on the inhibition of kallikrein and factor XIa, we conclude that heparin does not significantly affect the protease activity of purified contact activation factors or the activities expressed by these proteases in plasma.

Pixley, R. A., Schapira, M., and *Colman, R. W.*

Blood **66**:198-203, 1985.

Other support: American Heart Association, Southeastern Pennsylvania Chapter; National Institutes of Health Individual National Research Service Award; and Swiss National Science Foundation.

From the Thrombosis Research Center, Temple University School of Medicine, Philadelphia, and Division de Rhumatologie, Hôpital Cantonal Universitaire, Geneva, Switzerland.

THE REGULATION OF HUMAN FACTOR XIIa BY PLASMA PROTEINASE INHIBITORS

Studies of the inactivation of factor XIIa by plasma protease inhibitors in purified systems and in plasma were initiated to determine the relative importance of these inhibitors to the neutralization of factor XIIa. Factor XIIa was measured by the amidolysis of H-D-prolyl-L-phenylalanyl-L-arginine-p-nitroanilide dihydrochloride or by coagulant activity. C1 inhibitor (C1INH), α_2-antiplasmin (α_2AP), α_2-macroglobulin (α_2M), and antithrombin III (ATIII) inhibited factor XIIa with second-order rate constants of 2.2×10^5, 1.1×10^4, 5.0×10^3, and 1.3×10^3 $M^{-1}min^{-1}$. Factor XIIa activity was not affected by α_1-proteinase inhibitor. Incubation of ^{125}I-radiolabeled factor XIIa resulted in 1:1 stoichiometric complexes with C1INH (M_r 100,000), ATIII (M_r 125,000), and α_2AP (M_r 150,000 and 125,000) using sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Incubation of ^{125}I-Factor XIIa with α_2M resulted in a component of M_r 85,000 on a reduced sodium dodecyl sulfate-polyacrylamide gel, indicating that a subunit of factor XIIa was covalently bound to a proteolyzed portion of α_2M. The relative effectiveness of each inhibitor at plasma concentrations was 61:2:3:1 for C1INH, α_2AP, α_2M, and ATIII, respectively. Kinetic studies of the inactivation of purified factor XIIa added to various plasmas containing different concentrations of C1INH verified the predictions from the purified systems. Gel filtration of radiolabeled factor XIIa incubated with plasma confirmed that factor XIIa-C1INH was the major complex. Analysis by sodium dodecyl sulfate-polyacrylamide gel electrophoresis indicated that the complexes in plasma had the same molecular size as those with purified inhibitors. C1INH functions as the predominant inhibitor of factor XIIa in plasma.

Pixley, R. A., Schapira, M., and *Colman, R. W.*

The Journal of Biological Chemistry 260(3):1723-1729, 1985.

Other support: National Institutes of Health; American Heart Association, Southeastern Pennsylvania Chapter; and Swiss National Science Foundation.

From the Thrombosis Research Center and the Department of Medicine, Temple University School of Medicine, Philadelphia, and the Division de Rhumatologie, Hopital Cantonal Universitaire, Geneva, Switzerland.

EFFECT OF CLEAVAGE OF THE HEAVY CHAIN OF HUMAN PLASMA KALLIKREIN ON ITS FUNCTIONAL PROPERTIES

Human plasma kallikrein consists of an N-terminal heavy chain of molecular weight (mol wt) 52,000, linked by disulfide bonds to two light chain variants (mol wt 36,000 or 33,000). Although the active catalytic site of kallikrein resides on the C-terminal light chain, the role of the N-terminal heavy chain is less clear. We therefore studied an enzyme designated β-kallikrein, containing a single cleavage in the heavy chain (mol wt 28,000 + 18,000) and compared it to the enzyme, β-kallikrein, with an intact heavy chain. The rates of inactivation by C1 inhibitor of plasma α- and β-kallikreins were kinetically identical, as measured by residual amidolytic activity, after various times of incubation with the inhibitor. Both enzymes reacted completely with C1 inhibitor after 18 hours and formed identical C1 inhibitor-kallikrein complexes of mol wt 195,000. The rate of activation of factor XII by α-kallikrein and β-kallikrein was similar. In contrast, the rate of cleavage of high molecular weight kininogen (HMWK) by α-kallikrein was at least fivefold faster and the ratio of coagulant activity to amidolytic activity was fourfold greater than for β-kallikrein. Plasma α-kallikrein, at concentrations potentially achievable in plasma, induced aggregation of neutrophils, but β-kallikrein failed to elicit this response. In addition, human neutrophils that had been pretreated with cytochalasin B released 2.46 ± 0.10 $\mu g/10^7$ cells of elastase antigen, but β-kallikrein released only 0.25 ± 0.10 $\mu g/10^7$ cells. These observations suggest that cleavage of the heavy chaininfluences the rate of cleavage of HMWK and decreases its coagulant activity. Moreover, an intact heavy chain appears to be requisite to support the ability of kallikrein to aggregate neutrophils and release elastase.

Colman, R. W. et al.

Blood 65(2):311-318, 1985.

Other support: National Institutes of Health and the Swiss National Science Foundation.

the Thrombosis Research Center and the Hematology-Oncology Section, Department of Medicine, Temple University School of Medicine, Philadelphia, and the Graduate Hospital Research Center, Division de Rhumatologie, Hôpital Cantonal Universitaire, Geneva, Switzerland.

REGULATION OF THE COAGULANT ACTIVITY AND SURFACE BINDING OF HIGH MOLECULAR WEIGHT KININOGEN

Events in the formation of the enzymes in the intrinsic coagulation pathways can be conceptualized as three reaction clusters. The procofactors of the two later steps, factors VIII and V, are each proteolyzed by thrombin to active cofactors, factors VIIIa and Va, which are then bound to phospholipid micelles or platelet membranes. Each cofactor then serves as a "receptor" to which the enzymes, factors IXa and Xa, bind prior to activating the substrates, factor X and prothrombin. The cofactors, factors VIIIa and Va, are then inactivated by another enzyme protein, activated protein C. Our recent studies indicate that the procofactor of the contact system is similarly subject to activation by kallikrein to an active cofactor prior to binding to an activating surface.

The activation of the contact system is probably initiated by the binding of plasma factor XII to a negatively charged surface where autoactivation occurs for form factor XIIa. The substrates of factor XIIa, prekallikrein (PK) and factor XI, exist in bimolecular complexes with the contact system procofactor, high molecular weight kininogen (HMWK), which is required to transport them to an activating surface. Here, on the surface, these zymogens are converted to the active enzymes, kallikrein (K) and factor XIa, by activated factor XII. We have recently delineated the mechanism by which the binding of XII and HMWK on the surface is coordinated. XIIa first cleaves PK, which in turn converts the procofactor, HMWK, to an active cofactor, HMWKa, with the ability to bind to an activating surface and express coagulant activity by bringing PK and XI into proper apposition for cleavage by XIIa. XIIa then cleaves XI to XIa.

Colman, R. W., Silver, L. D., Purdon, A. D., Chang J. J., and Scott, C.F.

Transactions of the Association of American Physicians XCVII:113-123, 1984.

Other support: National Institutes of Health and Specialized Center of Research on Thrombosis.

From the Thrombosis Research Center and the Hematology-Oncology Section, Department of Medicine, Temple University School of Medicine, Philadelphia.

PLASMA PREKALLIKREIN ASSAY: REVERSIBLE INHIBITION OF C1 INHIBITOR BY CHLOROFORM AND ITS USE IN MEASURING PREKALLIKREIN IN DIFFERENT MAMMALIAN SPECIES

The assay of plasma prekallikrein requires activation of prekallikrein to kallikrein and sufficient inactivation of the plasma protease inhibitors of kallikrein to accurately measure the generated kallikrein activity. One method of elimination of the plasma protease inhibitors to kallikrein is to chemically pretreat the plasma. Methylamine has previously been employed to selectively inactivate α_2-macroglobulin. Our study examines the effect of sequential preincubation of plasma with chloroform and methylamine on the plasma prekallikrein assay. Chloroform was demonstrated to be a chemical inhibitor of purified C1 inhibitor, but α_2-macroglobulin was not. Chloroform inhibition of C1 inhibitor was not caused by precipitation of the protein into the interface between the water and organic solvent phase. Greater than 95% of C1 inhibitor antigen was recovered in the supernatant of chloroform-treated purified C1 inhibitor,

and chloroform-saturated buffer inhibited purified C1 inhibitor. Chloroform did not dissociate a preformed complex of kallikrein and C1 inhibitor, but its inhibition of C1 inhibitor was reversible. The addition of methylamine to plasma pretreated with chloroform in the plasma prekallikrein assay allowed for only a slight increase in the amount of kallikrein measured a 1 minute kaolin activation times, but provided for sustained measurement of activated prekallikrein when kaolin activation times were 5 to 7 minutes. Without chemical pretreatment, prekallikrein was not measurable in rabbit plasma. Both rabbit and pig plasma prekallikrein were measurable after exposure of the plasma to chloroform and methylamine, although the peak activation times and the contribution of each animal's protease inhibitors varied with the species. Our results show that chloroform is a reversible inhibitor of C1 inhibitor, and that the plasma prekallikrein assay in which it is used is useful for the measurement of prekallikrein in nonhuman mammalian plasma samples.

Schmaier, A. H., Gustafson, E., Idell, S., and *Colman, R. W.*

The Journal of Laboratory and Clinical Medicine 104(6):882-892, 1984.

Other support: National Heart, Lung and Blood Institute, American Heart Association, and National Institutes of Health.

From the Thrombosis Research Center, Hematology/Oncology and Pulmonary Sections, Department of Medicine, Temple University School of Medicine, Philadelphia.

THE EFFECT OF HIGH MOLECULAR WEIGHT KININOGEN ON SURFACE-ADSORBED FIBRINOGEN

High molecular weight kininogen (HMWK) plays an important role in altering the association of plasma fibrinogen with surfaces. Plasma initially deposits fibrinogen onto most materials, but on hydrophilic surfaces within 10 min adsorbed plasma fibrinogen cannot be detected on the surface by anti-fibrinogen antisera. However, using HMWK-deficient plasma, fibrinogen remains immunologically identifiable. The inter-relationship of adsorbed plasma fibrinogen with kininogen on hydrophilic surfaces is studied further using glass slides stained for protein with Coomassie Blue, and oxidized silicon crystal slices in an automated ellipsometer. On glass slides when plasma that is deficient in both low molecular weight kininogen (LMWK) and HMWK is reconstituted with HMWK (0.40 Units/ml), fibrinogen is no longer detected on the surface. This finding is specific for HMWK, since, when the same plasma is reconstituted with LMWK (220 μg/ml), the amount of fibrinogen detected on the surface is unchanged. The alteration of surface-adsorbed fibrinogen by HMWK is not due to plasmin-induced fibrinolysis, since it occurs in plasminogen-free plasma. In the ellipsometer, surface adsorption of normal plasma is associated with a significantly less ($p < 0.0005$) thick protein layer (1.99 $\pm$ 0.09 degree change in azimuth) than plasmas deficient in HMWK (2.32 $\pm$ 0.11). Using ellipsometry, HMWK in plasma is shown to shorten the time in which immunologically detectable surface-adsorbed fibrinogen was removed or altered. These studies in a whole plasma system present further evidence that HMWK specifically modifies the association of plasma fibrinogen with hydrophilic surfaces.

Schmaier, A. H., Silver, L., Adams, A. L., Fischer, G. C., Munoz, P. C. Vroman, L., and *Colman, R. W.*

Thrombosis Research 33:51-67, 1984.

Other supports: National Heart, Lung and Blood Institute, American Heart Association, a Temple University Biomedical Research Support Grant, and National Institutes of Health.

From the Hematology/Oncology Section and Thrombosis Research Center, Temple University School of Medicine, Philadelphia; and the Interface Laboratory, Veterans Administration Medical Center, Brookly, New York.

CHROMOSOMAL TRANSFER IN MOUSE ERYTHROLEUKEMIA CELLS SHOWS THAT TRANS REGULATION MEDIATES TISSUE SPECIFIC AND DEVELOPMENTALLY SPECIFIC PATTERNS OF GLOBIN GENE EXPRESSION

The data presented in this paper have suggested that the activation of embryonic globin genes in the K562 cell line arose through a single mutation which led to altered expression of a diffusible regulatory factor capable of activating embryonic globin genes. To test the hypothesis that embryonic globin gene activation had occurred in the K562 cell through altered expression of a trans regulatory factor, rather than a mutation in cis to the gene, it was decided to introduce the transcriptionally active zeta globin genes of the K562 cell into the tetraploid Friend mouse erythroleukemia cell in which adult but not embryonic globin gene expression occurs. In this study the authors also used restriction fragments length polymorphisms to show that both homologues of chromosome 16 which are present in the K562 cells contain alpha and zeta globin genes which behave in an identical manner when transferred to the mouse erythroleukemia cells. The results show that the mutation which led to the activation of human embryonic globin genes in the K562 cells is asyntenic to the zeta globin genes. These results also suggest that the K562 cell contains a trans regulatory factor which is competent to support the expression of the human embryonic globin genes.

Deisseroth, A. B.

In: Stamatoyannopoulof, G. and Nienhuis, A. (eds.): *Experimental Approaches for the Study of Hemoglobin Switching*. New York: Alan R. Liss, 1982, 293-303.

Other support: Veterans Administration and National Heart, Lung and Blood Institute.

From the Department of Medicine, University of California at San Francisco, Hematology/Oncology Unit, and the Veterans Administration Medical Center, San Francisco.

PLASMA PROTEIN AND LIVER mRNA LEVELS OF TWO CLOSELY RELATED MURINE α1-PROTEASE INHIBITORS DURING THE ACUTE PHASE REACTION

Plasma levels of α1-PI(T) and α1-PI(E), two closely related murine alpha 1-protease inhibitors, having affinities for trypsin and elastase, respectively, were compared to changes in specific liver mRNA levels after induction of the acute-phase reaction by subcutaneous injection of turpentine. In earlier, qualitative experiments, an increase in plasma levels of α1-PI(E) but not α1-PI(T) during the acute-phase reaction had been shown. It is now shown that stimulation of plasma α1-PI(E) levels reaches a maximum of 35-50% above baseline 12h after induction of the acute-phase response using either a functional or immunological assay to measure protease inhibitor activity. Consistent with earlier observations, little or no change in plasma levels of α1-PI(T) is

seen. Determination of mRNA levels in the mouse liver specific for α1PI(E) and α1-PI(T) was accomplished using a cell-free translation system followed by immunoprecipitation of the ^{35}S-labeled protease inhibitors. The apparent molecular weights of α1-PI(E) and α1-PI(T) synthesized *in vitro* are 42K and 46K, respectively. Apparent molecular weights of the native proteins in plasma are 55K and 65K. Unexpectedly, mRNA levels for both α1-PI(E) and α1-PI(T) were found to increase after induction of the acute-phase reaction. Maximal stimulation for both mRNAs was approximately 300% and occurred 9h after turpentine administration. Under these conditions, levels of translatable albumin mRNA in the mouse liver decreased to 40% of baseline in 6-9h.

Frazer, J. M., Nathoo, S. A., Katz, J., Genetta, T. L., and *Finlay, T. H.*

Archives of Biochemistry and Biophysics 39(1):112-119, 1985.

Other support: National Science Foundation.

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REGULATION OF PLASMA FACTOR XIII BINDING *IN VITRO*

The binding of plasma factor XIII to fibrinogen or fibrin that has been chemically or enzymatically induced to polymerize was studied. Factor XIII binding was assayed using a ^{3}H-putrescine incorporation assay and an ^{125}I-plasma factor XIII binding assay. More than 80% of the native and radiolabeled plasma factor XIII was bound to fibrin I formed by reptilase in EDTA, citrate, or heparin anticoagulated plasma. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of ^{125}I-plasma factor XIII bound to fibrin I or fibrin II formed by reptilase or thrombin in the presence of EDTA demonstrated the b_2-subunit remained bound to the a-chains or thrombin-cleaved a-chains. In the presence of calcium chloride and thrombin, the b_2-subunit dissociated and factor XIIIa was bound. Protamine sulfate caused fibrinogen polymerization in the absence of divalent cations and reduced both plasma factor XIII and immunologic fibrinogen levels. Fibrinogen polymerized by protamine sulfate bound plasma factor XIII and the a_2-subunit of ^{125}I-platelet factor XIII. Plasma factor XIII was also bound to sonicated non-cross-linked fibrin II in either normal plasma or afibrinogenemic plasma. Plasma levels of several coagulation proteins were unchanged after the addition of reptilase, protamine sulfate or sonicated fibrin to plasma. These results demonstrate that a specific binding site for the a_2-subunit of plasma factor XIII is present on polymerized fibrinogen, fibrin I and fibrin II. Furthermore, the presence of divalent cations, thrombin-cleavage of plasma factor XIII and release of fibrinopeptides A or B are not required for plasma factor XIII binding to polymerized fibrinogen and fibrin.

Greenberg, C. S., Dobson, J. V., and Miraglia, C. C.

Blood 66(5):1028-1034, 1985.

Other support: American Heart Association.

From the Departments of Medicine and Pathology, Duke University Medical Center, Durham, NC.

MEASUREMENT OF BLOOD COAGULATION FACTOR XIIIa FORMATION IN PLASMA CONTAINING GLYCYL-L-PROLYL-L-ARGINYL-L-PROLINE

A method is described to directly measure the formation of blood coagulation Factor XIIIa in platelet-poor plasma unmodified by heat. The synthetic peptide glycyl-L-prolyl-L-arginyl-L-proline, a fibrin-polymerization inhibitor, was used to prevent clotting of platelet-poor plasma. Plasma was diluted to a final concentration of 2.5% (v/v) in 0.1 M Tris-HCl, pH 8.5, buffer containing 25% glycerol, 5 mM calcium chloride, and 0.25 mM glycl-L-prolyl-L-arginyl-L-proline and then activated by thrombin (20 U/ml) for 15 min. The Factor XIIIa-catalyzed incorporation [^{3}H]putrescine into Hammersten casein was used to measure Factor XIIIa formation. The assay detected Factor XIIIa in 2.5 to 50 μl of thrombin-treated plasma. When purified Factor XIII was added to Factor XIII-deficient plasma, there was complete recovery of the Factor XIII added. Glycl-L-prolyl-L-arginyl-L-proline did not inhibit Factor XIIIa activity in thrombin-treated plasma or purified platelet Factor XIIIa. Glycerol stabilized Factor XIIIa activity in thrombin-treated plasma and buffer for 60 min. The presence of fibrinogen in plasma did not modify the assay results. The time course of thrombin-catalyzed Factor XIIIa formation in platelet-poor plasma containing glycyl-L-prolyl-L-arginyl-L-proline was directly measured using the assay.

Miraglia, C. C. and *Greenberg, C. S.*

Analytical Biochemistry 144:165-171, 1985.

Other support: National Institutes of Health.

From the Department of Medicine, Duke University Medical Center, Durham, NC.

THE EFFECT OF FIBRIN POLYMERS ON THROMBIN-CATALYZED PLASMA FACTOR XIIIa FORMATION

The effect of fibrin polymers on thrombin-catalyzed factor XIIIa formation was studied in afibrinogenemic plasma. Fibrin polymers derived from des A fibrinogen and des A,B fibrinogen increased six-fold the rate of thrombin-catalyzed factor XIIIa formation in the presence of EDTA. Calcium chloride accelerated factor XIIIa formation 14-fold in the presence of des A,B fibrinogen without increasing the rate of thrombin formation. Fibrinopeptides A and B had no effect on factor XIIIa formation in afibrinogenemic plasma. Des A,B fibrinogen reduced by 20- to 40-fold the thrombin concentration required to activate factor XIII. Glycyl-L-prolyl-L-arginyl-L-proline (gly-pro-arg-pro), a fibrin polymerization inhibitor, inhibited des A and des A,B fibrinog[illegible] from enhancing thrombin-catalyzed factor XIIIa formation. Gly-pro-arg-pro did not modify factor XIIIa formation in afibrinogenemic plasma and did not inhibit thrombin cleavage of the chromogenic substrate S-2238. These results demonstrate that fibrin polymers accelerate thrombin-catalyzed plasma factor XIIIa formation.

Greenberg, C. S. and Miraglia, C. C.

Blood 66(2):466-469,1985.

Other support: National Institutes of Health and American Heart Association.

From the Departments of Medicine and Pathology, Duke University, Durham, NC.

RADIOIMMUNOASSAY FOR NEUROPEPTIDE Y (NPY): CHROMATOGRAPHIC CHARACTERIZATION OF IMMUNOREACTIVITY IN PLASMA AND TISSUE EXTRACTS

A sensitive and specific radioimmunoassay was developed to determine the occurrence and concentration of neuropeptide Y (NPY) in plasma and tissue extracts. Furthermore, NPY-like immunoreactivity (NPY-Li) was characterized by means of three different chromatographic systems. The NPY antiserum used (Nl) did not cross-react with related peptides of the pancreatic polypeptide family except avian pancreatic polypeptide (1% cross-reactivity). Unextracted plasma contained high molecular weight proteins which interfered in the assay. Acid ethanol extraction removed this protein interference allowing a 90% recovery of NPY-Li. The content of NPY-Li in human plasma from healthy subjects was close to or below the detection limit (< 22 pmol/l). Sympathetic nerve stimulation in the cat increased the output of NPY-Li from the splenic vein suggesting the release of this peptide upon sympathetic activation. The major peak of NPY-Li in spleen extracts and splenic vein plasma co-eluted with synthetic porcine NPY and a minor peak with larger Stokes radius was also present. the present radioimmunoassay enables further studies on the physiological and pathophysiological role of NPY.

Theodorsson-Norheim, E., Hemsen, A. and *Lundberg, J. M.*

Scandinavian Journal of Clinical & Laboratory Investigation 45:355-365, 1985.

Other support: The Swedish Medical Research Council, the Karolinska Institute and the Astra Foundation.

From the Department of Clinical Chemistry, Karolinska Hospital, and the Department of Pharmacology, Karolinska Institute, Stockholm, Sweden.

IDENTIFICATION OF HUMAN PLATELET MEMBRANE FIBRINOGEN RECEPTORS BY IMMUNOCHEMICAL TECHNIQUES

The fibrinogen receptors of platelets were investigated with the use of three types of anti-platelet membrane antibodies and three types of platelets. We found that antisera raised in rabbits against membranes prepared from human intact, chymotrypsin- or pronase-treated platelets inhibited the fibrinogen-induced aggregations of ADP-stimulated intact platelets, chymotrypsin-treated platelets and pronase-treated platelets. These antisera also blocked the binding of ^{125}I-fibrinogen to ADP-stimulated intact, chymotrypsin-treated, and pronase-treated platelets. These results suggest that all three antisera blocked the interaction of fibrinogen with its receptor on the surface of the three types of platelets studied. Fibrin clot retraction by intact platelets was also inhibited by these three antibodies indicating an important role of platelet membrane proteins in clot retraction. As demonstrated by techniques using ^{125}I-surface labeling, *Staphylococcus aureus* immunoprecipitation, SDS-polyacrylamide gel electrophoresis and autoradiography, anti-intact platelet membrane antibody immunoprecipitated the membrane glycoproteins GPIIb, GPIII and a protein with an apparent molecular weight of 66,000 from detergent solubilized surface ^{125}I-iodinated chymotrypsin-treated platelets. Anti-chymotrypsin and anti-pronase-treated platelet membrane antisera immunoprecipitated mostly GPIII and the 66,000 molecular weight protein from detergent solubilized surface ^{125}I-iodinated chymotrypsin-treated platelets.

The 66,000 Mr protein was not found on the surface of intact (unstimulated) platelets which do not bind ^{125}I-fibrinogen and are not aggregated by fibrinogen without the prior addition of ADP. The ability of anti-platelet membrane antibodies to block fibrinogen-induced platelet aggregation and fibrinogen binding to platelets correlated with their ability to immunoprecipitate a 66,000 Mr protein from the platelet surface. It is proposed that the 66,000 Mr protein may be the fibrinogen binding domain of GPIII which becomes permanently exposed on the surface of chymotrypsin and pronase-treated platelets following proteolysis and which becomes exposed upon stimulation of intact platelets by agents such as ADP.

Kornecki, E., Tuszynski, G. P., and *Niewiarowski, S.*

Hematologia 17(3):387-398, 1984.

Other support: National Institutes of Health and National Research Service.

From the Thrombosis Research Center, Temple University Health Sciences Center, Philadelphia.

MEMBRANE FLUIDITY AND PLATELET FIBRINOGEN RECEPTOR EXPOSURE BY PROTEOLYTIC ENZYMES

Incubation of platelets with pronase or chymotrypsin results in the exposure of fibrinogen receptors. We determined that these enzymes did not affect the membrane fluidity as evaluated by the depolarization of the fluorescence of 1,6-diphenyl-1,3,5 hexatriene (DPH). There was no significant difference in either the depolarization or in its temperature dependence for control, pronase or chymotrypsin-treated platelets. Thus, it can be concluded that the exposure of fibrinogen receptors on the platelet surface by proteolytic enzymes does not depend on the changes of membrane fluidity. We also propose that the proteolytic enzymes do not cause a major alteration in the extent of protein chains embedded in the lipid layers of the platelet membranes.

Simons, E., Whitin, J. C., Morinelli, T. A., Stewart, G. J., and *Niewiarowski, S.*

Thrombosis Research 39:91-96, 1985.

Other support: National Institutes of Health.

From the Thrombosis Research Center and Department of Physiology, Temple University Health Sciences Center, Philadelphia, and the Department of Biochemistry, Boston University School of Medicine, Boston.

AGGREGATION OF CHYMOTRYPSIN-TREATED THROMBASTHENIC PLATELETS IS MEDIATED BY FIBRINOGEN BINDING TO GLYCOPROTEINS IIb AND IIIa

Previous experiments demonstrated that chymotrypsin, but not adenosine diphosphate (ADP), exposed fibrinogen binding sites on platelets from patients with Glanzmann's thrombasthenia. Three of these patients have been reexamined, and previous observations were confirmed. The quantity of iodine 125-labeled glycoprotein IIb (GPIIb) and glycoprotein IIIa (GPIIIa) on the platelets of these patients was

considerably less than normal but was detectable by immunoprecipitation, sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and autoradiography. The amount of residual GPIIb and GPIIIa as measured by binding studies with radiolabeled monoclonal antibodies was between 3% and 12% of the normal value. Platelet suspensions from these patients did not aggregate with fibrinogen and did not bind ^{125}I-fibrinogen on stimulation with ADP. However, incubation of these platelets with chymotrypsin or pronase resulted in fibrinogen binding and platelet aggregation. Monocolonal antibodies specific for the GPIIb-GPIIIa complex blocked both the fibrinogen binding and the aggregation of enzyme-treated platelets. The treatment of washed platelets of a fourth thrombasthenic patient with ADP or with chymotrypsin failed to result in fibrinogen binding and aggregation. However, the level of GPIIb and GPIIIa on these platelets as measured by a Western blot technique and by monoclonal antibody binding amounted to < 0.35% to 0.5% of normal values. In conclusion, fibrinogen binding sites exposed on thrombasthenic platelets by chymotrypsin are derived from GPIIb-GPIIIa molecules. Aggregation of chymotrypsin-treated thrombasthenic platelets by fibrinogen appears to represent a sensitive test for detection of functionally active GPIIb-GPIIIa complex on the platelet surface.

Niewiarowski, S. et al.

Journal of Laboratory and Clinical Medicine 106(6):651-660, 1985.

Other support: National Institutes of Health and the American Heart Association.

From the Thrombosis Research Center and Department of Physiology, Temple University Health Sciences Center, Philadelphia; the Hematology/Oncology Section, Department of Medicine, University of Pennsylvania School of Medicine, Philadelphia; and the Hôpital Lariboisière, Paris, France.

IDENTIFICATION OF PI^{A1} ALLOANTIGEN DOMAIN ON A 66 kDa PROTEIN DERIVED FROM GLYCOPROTEIN IIIa OF HUMAN PLATELETS

Incubation of platelets with chmyotrypsin leads to the exposure of fibrinogen receptors and to the appearance of a 66 kDa membrane component on the surface of platelets. Both glycoprotein IIIa (GP IIIa) and a 66 kDa component were precipitated from detergent extracts of solubilized, surface radiolabeled chymotrypsin-treated platelets by human anti-PI^{A1} antisera. Moreover, the presence of the PI^{A1} antigen was identified on GP IIIa (but not on GP IIb) and on a 66 kDa protein by means of immunoblot procedures using platelet Triton X-114 extracts and these purified proteins. Anti-PI^{A1} antiserum did not recognize GP IIIa on the surface of intact (untreated) platelets nor the 66 kDa protein on the surface of chymotrypsin-treated platelets of PI^{A1}-negative individuals. The present data demonstrate directly that the 66 kDa protein is derived from GP IIIa and contains the PI^{A1} alloantigen.

Kornecki, E., Chung, S.-Y, Holt, J. C., Cierniewski, C. S., Tuszynski, G. P., and *Niewiarowski, S.*

Biochemica et Biophysica Acta 818:285-290, 1985.

Other support: National Institutes of Health and the National Heart, Lung and Blood Institute.

From the Thrombosis Research Center, Temple University Health Science Center, Philadelphia, and the Department of Psychiatry, Neurosciences Research Unit, University of Vermont, Burlington.

THE INTERACTION OF HUMAN PLATELET THROMBOSPONDIN WITH FIBRINOGEN: THROMBOSPONDIN PURIFICATION AND SPECIFICITY OF INTERACTION.

Human platelet thrombospondin (TSP) was purified to homogeneity by chromatography on fibrinogen coupled to cyanogen bromide-activated Sepharose. The yield of TSP was 1.3 mg or approximately 22% of that present in platelet-rich plasma as determined by radioimmunoassay. It analyzed on discontinuous sodium dodecyl sulphate gels as a single band having apparent molecular weights of 180,000 and ≥ 400,000 under reducing and nonreducing conditions, respectively. Amino acid analysis gave results similar to previously published values. Antibodies raised in rabbits were monospecific as evaluated by radioimmunoassay. In double immunodiffusion tests, these antibodies gave one line of identity against TSP purified by this procedure and TSP purified by published procedures, confirming the identity of the material isolated. The protein possesses no lectin-like activity.

The specificity of the TSP-fibrinogen interaction was investigated. TSP binding to fibrinogen-Sepharose occurred in the presence of EDTA, indicating that calcium and magnesium ions are not required for interaction of TSP with fibrinogen. The binding of TSP to fibrinogen-Sepharose was quantitatively blocked by pretreatment with an antibody to the cyanogen bromide cleavage fragment composed of residues 241-476 of the carboxyl-terminal end of the α chain of fibrinogen. Antibodies against the D and E domains of fibrinogen had no effect on the binding. Excess fibrinogen (30 mg/ml) added to platelet extract quantitatively inhibited binding of TSP to fibrinogen-Sepharose. TSP preferentially bound to uncross-linked fibrin, suggesting that the TSP-fibrinogen binding site is unavailable in cross-linked fibrin. These results indicate that TSP binds specifically to immobilized fibrinogen or uncross-linked fibrin through determinants present in the carboxyl-terminal portion of the α chain and that these interactions do not require calcium or magnesium ions.

Tuszynski, G. P., Srivastava, S., Switalska, H. I., Holt, J. C., Cierniewski, C. S., and *Niewiarowski, S.*

The Journal of Biological Chemistry 260(2):12240-12245, 1985.

Other support: U. S. Department of Health and Human Services and the American Heart Association, Pennsylvania Keystone Affiliate.

From the Thrombosis Research Center and the Department of Physiology, Temple University School of Medicine, Philadelphia.

LOSS OF FIBRINOGEN RECEPTORS FROM THE PLATELET SURFACE DURING SIMULATED EXTRACORPOREAL CIRCULATION

In vitro recirculation of fresh human heparinized blood in an extracorporeal circuit with a membrane oxygenator decreased fibrinogen-induced platelet aggregation and diminished the number of fibrinogen receptors and glycoprotein IIb/IIIa (GPIIb/GPIIIa) antigenic sites on the platelet surface. In seven experiments, the mean ± SD K_m value for fibrinogen (i.e., molar concentration of fibrinogen required to cause 50% of the maximal rate of aggregation) was 1.58×10^{-7} mol/L ± 0.68×10^{-7} mol/L. After recirculation, this value increased to 3.8×10^{-7} mol/L ± 1.94×10^{-7} mol/L ($P \leq 0.025$).

The maximal aggregation rate of chymotrypsin-treated platelets decreased by 40% after 2 hours of recirculation ($P \leq 0.025$). The number of fibrinogen receptors on platelets, which were treated with chymotrypsin after recirculation, decreased from 41,370 ± 24,000 to 13,230 ± 10,230/platelet under the same conditions ($P \leq 0.025$). The number of antigenic sites for monoclonal antibody reacting with GPIIb/GPIIIa complex of adenosine diphosphate-stimulated platelets decreased from 34,200 ± 5,940 to 19,500 ± 9,680/platelet after recirculation ($P \leq 0.025$). Prostaglandin E_1 (0.3 μmol/L) in the perfusion circuit preserved the ability of platelets to react with fibrinogen. In conclusion, the loss of fibrinogen receptors from the surface of platelet membranes results from the interaction of platelets with the surfaces of perfusion circuits.

Musial, J., *Niewiarowski, S.*, Hershock, D., Morinelli, T. A., Colman, R. W., and Edmunds, L. H. Jr.

Journal of Laboratory and Clinical Medicine 105:514-522, 1985,

Other support: National Institutes of Health.

From the Department of Cardiovascular Surgery, University of Pennsylvania Hospital and the Thrombosis Research Center, Temple University Health Sciences Center, Philadelphia.

RADIOIMMUNOASSAY OF HUMAN PLATELET THROMBOSPONDIN: DIFFERENT PATTERNS OF THROMBOSPONDIN AND β-THROMBOGLOBULIN ANTIGEN SECRETION AND CLEARANCE FROM THE CIRCULATION

A method for radioimmunoassay of human thrombospondin was developed. Monospecific precipitating anti-human thrombospondin antibody was raised in rabbits after injection of thrombospondin purified by fibrinogen-agarose chromatography and preparative sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The linear portion of the thrombospondin radioimmunoassay standard curve was 0.5 to 20 ng/ml. Normal platelets and platelet-poor plasma contained 28,900 ± 14,500 ng thrombospondin per 10^9 platelets and 60.6 ± 10.7 ng/ml (mean ± SD), respectively. Using radioimmunoassays for β-thromboglobulin and thrombospondin antigens, we compared platelet location and secretion of these proteins. Both antigens shared similar distributions in platelet subcellular fractions with the largest amount localized to platelet α-granules. With thrombin (0.25 U/ml) as a platelet agonist, 62.4% and 19.5% of total β-thromboglobulin and thrombospondin, respectively, were secreted from suspensions of washed human platelets. Because only 20% of the total platelet thrombospondin was secreted, further studies were initiated to determine whether the remaining thrombospondin became localized on the activated platelet membrane. ^{125}I-Fab antithrombospondin specifically bound to activated platelets but not to unstimulated platelets. In contrast, ^{125}I-Fab anti-β-thromboglobulin did not bind to activated platelets. Plasma clearance of human β-thromboglobulin (half-life fast 7.6 minutes, slow 56.6 minutes) and of human thrombospondin (half-life fast 29.9 minutes, slow 190 minutes) followed a biphasic exponential curve. In conclusion, both β-thromboglobulin and thrombospondin are located in platelet α-granules, but they show a different pattern of secretion and expression on the platelet membrane and plasma clearance.

Switalska, H. I., *Niewiarowski, S. et al.*

Journal of Laboratory and Clinical Medicine 106:690-700, 1985.

Other support: National Institutes of Health and the American Heart Association.

From the Thrombosis Research Center, Departments of Physiology and Medicine, Temple University Health Sciences Center, Philadelphia.

INTERACTION OF CHRONIC CIGARETTE AND ETHANOL USE ON MYOCARDIUM

Chronic cigarette use is common in persons who habitually use other cardioactive agents that have been causally associated with heart disease. This study was undertaken to determine if cigarette use intensifies the abnormalities of myocardial function and composition observed in experimental alcoholism over an 18-month period. Young adult male beagles with tracheostomy were divided into four groups. There were 10 controls (group 1); 9 smoked seven cigarettes per day (group 2); 7 were fed ethanol as 20% of calories (group 3), and 6 received both ethanol and cigarettes (group 4). After a period of 18 months, left ventricular function was assessed under anesthesia. Heart rate, left ventricular end-diastolic pressures and volumes (indicator dilution) did not differ in the four groups. An index of contractility derived by normalizing peak dP/dt for pre- and after-load was reduced significantly below the level of 2.4 $\pm$ 0.7 cm/s in control dogs to 1.41 $\pm$ 0.35 in group 2, 1.1 $\pm$ 0.38 in group 3, and1.28 $\pm$ 0.17 in the ethanol cigarette group (each $p \leq 0.002$). Arterial pressures were moderately elevated above group 1 in all three experimental groups without evidence of left ventricular hypertrophy. In contrast to smoking, which elicited no abnormalities of myocardial cation composition, ethanol reduced myocardial potassium and sodium in group 3 without a gain of water content. In group 4, no further decline of tissue cations was observed. Thus, cigarette use when combined with ethanol over a relatively long period, produced no greater myocardial abnormalities than ethanol alone and may not be essential to the genesis of cardiomyopathy in alcoholics.

Ahmed, S. S., Torres, R., and *Regan, T. J.*,

Clinical Cardiology 8(3):129-136, 1985.

From the Division of Cardiovascular Diseases, Department of Medicine and Dentistry of New Jersey-New Jersey Medical School, Newark.

ISOLATION AND CULTURE OF PULMONARY ENDOTHELIAL CELLS

Methods for isolation, identification and culture of pulmonary endothelial cells are now routine. In the past, methods of isolation have used proteolytic enzymes to detach cells; thereafter, traditional methods for cell passaging have used trypsin/EDTA mixtures. Cells isolated and passaged using proteolytic enzymes have been useful in establishing the field and in verifying certain endothelial properties. However, there is a growing awareness of the role of endothelial cells in processing vasoactive substances, in responding to hormones and other agonists, and in cell-cell interactions with other cell types of the vascular wall, with blood cells and with cellular products. Consequently, a new requirement has arisen for cells *in vitro* that maintain the differentiated properties of their counterparts *in vivo*. The deleterious effects of trypsin and other proteolytic enzymes commonly used in cell culture on surface structures of endothelial cells such as enzymes, receptors and junctional proteins, as well as on extracellular layers such as the glycocalyx or "endothelial fuzz," have led to the development of methods that avoid use of proteolytic enzymes at both the isolation step and during subsequent subculture. This

chapter describes traditional methods for isolating pulmonary endothelial cells but emphasizes newer approaches using mechanical harvest and scale-up using microcarriers. The new methods allow maintenance of long-term, large-scale cultures of cells that retain the full complement of surface properties and that maintain the cobblestone monolayer morphology and differentiated functional properties. Methods for identification of isolated cells are therefore also considered as methods for validation of cultures during their *in vitro* lifespan.

Ryan, U. S.
Environmental Health Perspectives 56:103-114, 1984.

Other support: National Institutes of Health and the John A. Hartford Foundation.

From the Department of Medicine, University of Miami School of Medicine, Miami, FL.

VARICOSE VEINS AS A SOURCE OF ADULT HUMAN ENDOTHELIAL CELLS

Endothelial cells can be harvested from segments of adult human saphenous vein in a varicose condition removed from patients having single or bilateral vein ligation and stripping. The cells are harvested by scraping with a scalpel, seeded on to gelatin coated or Primaria flasks and are passaged by removal with a rubber policeman. The cells cultured in this manner are maintained in a growth medium that is not supplemented with growth factors. The cells grow with a cobblestone monolayer morphology, possess angiotensin converting enzyme activity and react with antibodies to Factor VIII antigen. The cells fluoresce brightly after reaction with monoclonal antibodies specific for human endothelial cells. Thus, stripped varicose vein segments provide a readily available source of endothelial cells.

Ryan, U. S. and White, L. A.

Tissue & Cell 17(2):171-176, 1985.

Other support: National Institutes of Health.

From the Department of Medicine, University of Miami School of Medicine, Miami, FL.

A POSSIBLE REGULATORY ROLE OF SQUALENE EPOXIDASE IN CHINESE HAMSTER OVARY CELLS

Growth of Chinese hamster ovary cells (CHO) in the presence of 20% lipid depleted serum (LDS) for only 2hr results in an increase in the synthesis of [^{14}C] sterols from [^{14}C] mevalonate and from [^{14}C] squalene compared with cells grown under normal growth conditions in the presence of 10% fetal calf serum (FCS). This enhanced sterol synthesis increases with time of exposure of the cells to LDS. However, exposing these cells for time periods up to 42.52hr to a growth medium containing 20% LDS did not result in enhanced [^{14}C] sterol synthesis from [^{14}C] 2,3,-oxidosqualene. Incubation of these cells with [^{14}C] mevalonate resulted in the accumulation of [^{14}C] squalene regardless of the presence of either LDS or FCS. These results suggest that squalene epoxidase is a regulatory enzyme in the cholesterol biosynthetic pathway in CHO.

Eilenberg, H. and *Shechter, I.*

Lipids 19(7):539-543, 1984.

From the Department of Biochemistry, the George S. Wise Faculty of Life Sciences, Tel Aviv University, Israel.

UPTAKE OF HIGH-DENSITY LIPOPROTEIN-ASSOCIATED APOPROTEIN A-I AND CHOLESTEROL ESTERS BY 16 TISSUES OF THE RAT *IN VIVO* AND BY ADRENAL CELLS AND HEPATOCYTES *IN VITRO*

The uptake of high-density lipoprotein (HDL)-associated apolipoprotein A-I (apo A-I) and cholesterol esters was estimated in 16 tissues of the rat using rat HDL double labeled with nondegradable tracers; covalently attached ^{125}I-tyramine-cellobiose traced apo A-I, and [^{3}H]cholesteryl linoleyl ether traced cholesteryl esters. Both labels remained associated with the HDL fraction in the plasma, adequately traced their unlabeled counterparts, and were well trapped at their sites of uptake.

Cholesterol ether was taken up at a greater fractional rate than apo-A-I by adrenal, ovary and liver: 7-fold, 4-fold, and 2-fold greater, respectively. The rates of uptake of cholesterol ether and apo-A-I were about equal in the other tissues (except kidney). The disproportionate uptake of HDL cholesterol ether relative to HDL apo A-I was also observed in primary cultures of rat adrenal cells and hepatocytes. Uptake of both moieties in both cell types showed saturability. Both the absolute rate of uptake of [^{3}H]cholesterol ether and the ratio of ether uptake to apo-A-I uptake were greater in adrenal cells than in hepatocytes, consonant with the *in vivo* observations. Very similar results were obtained using HDL biologically labeled with [^{3}]cholesterol *esters*. The disproportionate uptake of [^{3}H]cholesterol ether was not significantly decreased by depletion of apo-E from the HDL, nor by reductive methylation of the apo-E to block its recognition by receptors. However, apo-A-I uptake was decreased, suggesting that apo-E mediates the uptake of particles containing apo-A-I but does not contribute to the disproportionate uptake of [^{3}H]cholesteryl ether.

Glass, C., Pittman, R. C., Civin, M., and *Steinberg, D.*

The Journal of Biological Chemistry 260(2):744-750, 1985.

Other support: National Heart Lung and Blood Institute and National Institute of General Medical Sciences.

From the Department of Medicine, Division of Endocrinology and Metabolism, University of California at San Diego, La Jolla.

ROLE OF THE LOW DENSITY LIPOPROTEIN RECEPTOR IN PENETRATION OF LOW DENSITY LIPOPROTEIN INTO RABBIT AORTIC WALL

The present study was designed to determine whether binding of low density lipoprotein (LDL) to endothelial LDL receptors contributes significantly to the penetration of LDL into normal rabbit aorta. Initial flux rate was used as a measure of uptake of LDL. Reductive methylation of LDL is known to block its recognition by the LDL receptor. Therefore, the difference in flux rates of native LDL and reductively methylated LDL (methyl-LDL) was assumed to represent the receptor-dependent uptake. Native LDL and methyl-LDL were labeled with different isotopes (^{125}I or ^{131}I) and both were injected simultaneously into the same rabbit. After 30 to 60 minutes, trichloracetic acid-precipitable counts were determined in aortic specimens. The initial flux rates, expressed as plasma clearance (nl/g/hr), were 1787 for native LDL and 1924

for methyl-LDL. The difference was not significant, which suggests that the flux of LDL into the aorta is not significantly dependent upon, or regulated by, endothelial LDL receptors, but is mediated by other mechanisms.

Wiklund, O., Carew, T. E. and *Steinberg, D.*

Arteriosclerosis 5:135-141, 1985.
Other support: National Heart Lung and Blood Institute and the National Institutes of General Medical Sciences.

From the Department of Medicine, University of California at San Diego, La Jolla.

CONTRIBUTION OF ACTIN TO THE STRUCTURE OF THE CYTOPLASMIC MATRIX

The realization that actin is a constituent of nonmuscle cells (1) and the identification of actin filaments in the periphery of such cells (2) opened up a new world of molecular biology. For the first time, it became possible to explain in detail a body of phenomena described over two centuries concerning important aspects of cell shape, movement, and consistency. Although actin comprises only one of several intracellular fiber systems of cells, it is an extremely important one. This essay briefly reviews some present concepts of the contribution of actin to the "cytoplasmic matrix."

Stossel, T. P.

The Journal of Cell Biology 99(1):155-215, Pt.2, July, 1984.

Other support: U. S. Public Health Service, the Muscular Dystrophy Association, and the Edwin S. Webster Foundation.

From the Hematology/Oncology Unit, Massachusetts General Hospital, Department of Medicine, Harvard Medical School, Boston.

NONMUSCLE ACTIN-BINDING PROTEINS

Because the workings of actin-binding proteins depend upon the characteristics of actin itself, the first section of this article summarizes current knowledge of actin assembly. The ensuing review of actin-binding proteins classifies these proteins according to their functions, as has been done previously (Schliwa 1981; Weeds 1982; Craig & Pollard 1983; Stossel 1984). There are actin-binding proteins that bind predominantly to actin monomers and inhibit their self-assembly into filaments. Other proteins bind to one or more actin monomers when they are in the form of filaments, either to the ends of the filaments or in some instances to monomers within the filament, causing the filament to break. They then remain bound to one end of the fractured filament. Still other proteins bind [illegible] to actin monomers in different filaments, which causes the actin filaments to be cross-linked into particular conformations relative to one another. The word "predominant" is very important here because as work progresses on particular actin-binding proteins, their functions tend to become less "pure" and more complex. This review emphasizes biochemical data concerning the structural and functional properties of actin-binding proteins and their interactions with actin *in vitro*. Some effort is made, however, to speculate on how these interactions might operate *in vivo*.

Stossel, T. P. et al.

Annual Review of Cell Biology 1:353–402, 1985.

Other support: U. S. Public Health Service and the Edwin S. Webster Foundation.

From the Hematology-Oncology Unit, Massachusetts General Hospital, Department of Medicine, Harvard Medical School, Boston.

COAGULANT ACTIVITIES OF PLATELETS IN CORONARY ARTERY DISEASE

Platelets have been implicated in the pathogenesis of coronary artery disease, and a number of studies have examined platelet function and coagulation parameters in patients with such disease. We have examined platelet coagulant activities, volumes, and aggregate ratios in 23 patients with chest pain, seven of whom had normal coronary angiograms (group I) and 16 of whom had angiographically proven coronary artery disease (Group II). There were no significant differences in the mean values for platelet volume or platelet aggregate ratios between the two groups. The platelet coagulant activities concerned with initiation and the early stages of intrinsic coagulation were significantly increased in patients in group II as compared with those in group I. No significant differences were noted between the two groups with respect to prothrombin time, partial thromboplastin time, and plasma levels of fibrinogen and coagulation factors V and VIII. However, the mean activity in plasma of antithrombin III (but not the level of antithrombin III antigen) was significantly lower in patients of group II compared with group I. Overall, our observations provide evidence for an enhanced contribution to the intrinsic coagulation system in patients with coronary artery disease. The platelet coagulant hyperactivity noted in these patients may reflect a role of platelets in the pathogenesis of coronary artery disease or may be secondary to the underlying arterial disease.

Rao, A. K., *Walsh, P. N. et al.*

Circulation 69(1):15–21, 1984.

Other support: Ciba-Geigy Corporation and U. S. Department of Health and Human Services.

From the Thrombosis Research Center and the Cardiology Section, Department of Medicine, Temple University Health Sciences Center, Philadelphia.

ACTIVATION OF COAGULATION FACTORS ON THE SURFACE OF PLATELETS

Platelet physiology and coagulation biochemistry have traditionally been studied and conceptualized as separate and distinct entities. This brief summary of platelet-coagulant protein interactions presents an alternative viewpoint which emphasizes the effects of platelets in coagulation and the effects of coagulation proteins on platelet physiology. These interrelationships are so intimate and important that any consideration of the hemostasis mechanism that fails to take them into account is meaningless.

Walsh, P. N.

Haematologia 17(1):55–66, 1984.

Other support: Public Health Service.

From the Thrombosis Research Center, Department of Medicine, Temple University School of Medicine, Philadelphia.

PLATELET COAGULANT ACTIVITIES IN DIABETES MELLITUS: EVIDENCE FOR RELATIONSHIP BETWEEN PLATELET COAGULANT HYPERACTIVITY AND PLATELET VOLUME

Platelets have been shown to be capable of initiating and promoting some of the reactions in intrinsic coagulation and have been implicated in the pathogenesis of vascular lesions in diabetes mellitus. We have examined the contribution of platelets to the coagulation reactions in 39 patients having diabetes mellitus with and without retinopathy [background retinopathy (BR) or proliferative retinopathy (PR)]. 18 patients with nonvascular eye diseases (patient controls), and 38 normal subjects. In comparison with control patients, significant elevations were noted in four of the five assays for platelet coagulant activities in diabetic patients with PR and in three of five assays in patients with BR. Thus, platelet coagulant hyperactivity is present in diabetics with retinopathy. Significant elevations were noted in two of the assays in patients without retinopathy (NR), suggesting that platelet coagulant hyperactivity may not necessarily be secondary to the vasculopathy. The predominant abnormalities noted were in assays reflecting the contribution of platelets to the early stages of intrinsic coagulation rather than to later stages of prothrombin activation. In addition, the mean platelet volume was found to be significantly greater in patients with PR and BR but not NR than in normal controls. A positive linear correlation ($r = 0.81$) was noted between the mean of the coagulant activities and platelet volume in diabetic subjects, suggesting a hitherto undescribed relationship between platelet coagulant activities and platelet volume.

Rao, A. K., Goldberg, R. E. and *Walsh, P. N.*

The Journal of Laboratory and Clinical Medicine 103(1):82-92, 1984.

Other support: National Institutes of Health.

From the Thrombosis Research Center and Department of Medicine, Temple University School of Medicine, and Retinal Vascular Unit, Wille Eye Hospital, Philadelphia.

THE POSSIBLE ROLE OF PLATELETS IN BYPASSING THE CONTACT PHASE OF BLOOD COAGULATION

Data presented herein and previously support an active role for platelets in promoting the interaction and activation of the coagulation proteins of the contact phase of intrinsic coagulation. The platelet membrane, activated by ADP collagen or thrombin, can promote the proteolytic activation of factor XII to factor XIIa in the presence of kallikrein and high molecular weight kininogen. The zymogen factor XI associates with high molecular weigh kininogen in plasma and becomes bound to a site on the membrane of thrombin or collagen activated platelets. Thereafter, platelet bound factor XI can be proteolytically activated to factor XIa either in the presence of factor XIIa or in the presence of kallikrein. These observations could explain the absence of bleeding complications in patients with factor XII deficiency. In addition, platelets contain a molecule which has a higher molecular weight than plasma factor XI and possibly consists

of a tetramer of four identical subunits of 52,000 daltons each of which is functionally and immunologically similar to plasma factor XI. Since this molecule is present in the platelets of patients with severe plasma factor XI deficiency and no evidence of bleeding, we postulate that platelet factor XI can substitute for plasma factor XI in hemostasis and possibly account for the considerable variability in clinical severity observed in patients with factor XI deficiency.

Walsh, P. N., Tuszynski, G. P., Greengard, J. S., and Griffin, J. H.

Haematologia 17(2):169-178, 1984.

Other support: U. S. Department of Health, Education and Welfare.

From the Thrombosis Research Center, Temple University School of Medicine, Philadelphia, and Scripps Research Institute, La Jolla, CA.

ASSOCIATION OF FIBRIN WITH THE PLATELET CYTOSKELETON

The authors have previously postulated that surface membrane proteins become specifically associated with the internal platelet cytoskeleton upon platelet activation. Four lines of evidence support this general hypothesis since we now show that platelet surface receptors for fibrin become specifically associated with the platelet Triton-insoluble cytoskeletons. 1) Fibrin was detected immunologically in the washed Triton-insoluble cytoskeletons of thrombin-activated platelets under conditions where fibrin polymerization and resultant precipitation were blocked with Gly-Pro-Arg-Pro, a synthetic peptide that inhibits polymerization of fibrin monomer. 2) Radiolabeled fibrin bound to thrombin-activated platelets and became associated with the cytoskeleton. 3) The amount of radiolabeled fibrin bound to thrombin-activated thrombasthenic platelets and their cytoskeletons amounted to about 20% of the fibrin bound to thrombin-activated control platelets and their cytoskeletons. 4) The association of fibrin with cytoskeletons and with the platelet surface was nearly quantitatively blocked by an antibody prepared against cytoskeletons (anti-C), an antibody against isolated membranes of Pronase-treated platelets (anti-M1), and a monoclonal antibody to the platelet surface glycoprotein complex, $GPII_b$-GPIII (anti-GPIII). These antibodies blocked ADP and thrombin-induced platelet aggregation as well as thrombin-induced clot retraction. Analysis of the immunoprecipitates obtained with anti-C, Anti-M1, and anti-GPIII from detergent extract of ^{125}I-surface labeled platelets revealed that these antibodies recognized $GPII_b$-GPIII. These data suggest that thrombin activation of platelets results in the specific association of fibrin with the platelet cytoskeleton, that this association may be mediated by the $GPII_b$-GPIII complex, and that these mechanisms may play an important role in platelet aggregation and clot retraction induced by thrombin.

Tuszynski, G. P., Kornecki, E., Cierniewski, C., Knight, L. C., Koshy, A., Srivastava, S., Niewiarowski, S., and *Walsh, P. N.*

The Journal of Biological Chemistry 259(8):5247-5254, 1984.

Other support: U. S. Department of Health and Human Services.

From the Thrombosis Research Center, Temple University School of Medicine, Philadelphia.

AMIDOLYTIC ASSAY OF HUMAN FACTOR XI IN PLASMA: COMPARISON WITH A COAGULANT ASSAY AND A NEW RAPID RADIOIMMUNOASSAY

The traditional coagulant assay for plasma factor XI suffers from a relatively high coefficient of variation, the need for rare congenitally deficient plasma, and a poor correlation between precision and sensitivity. We have developed a simple functional amidolytic assay for factor XI in plasma using the chromogenic substrate PyrGlu-Pro-Arg-*p*-nitroanalide (S-2366). After inactivation of α_1-antitrypsin, C1 inhibitor, and other plasma protease inhibitors with $CHCl_3$, plasma was incubated with kaolin, in the absence of added calcium, which limited the enzymes formed to those dependent on contact activation. Soybean trypsin inhibitor was used to mimize the action of kallikrein on the substrate. Once the reaction was complete, corn trypsin inhibitor was used to inactive factor XIIa, the enzyme generated by exposure of plasma to negatively charged surfaces which had activated the factor XI. The assay is highly specific for factor XI, since plasma total deficient in that zymogen yielded only 1%-3% of the enzymatic activity in normal plasma under identical conditions. The requirements for complete conversion of factor XI to XIa in plasma within 60 min were, respectively, factor XII, 0.6 U/ml. Prekallikrein was not an absolute requirement for complete activation but did accelerate the reaction. The intraassay coefficient of variation was 3.4%, and the mean of 35 normal plasmas was 1.00 U ± 0.24 SD. In addition, a new rapid radioimmunoassay was devised using staphylococcal protein A as the precipitating agent for a complex of factor XI antigen with monospecific rabbit antibody. The mean was 1.01 U ± 0.30 SD. The correlation coefficients for amidolytic *versus* coagulant and amidolytic *versus* radioimmunoassay were r = 0.95 for the former and 0.96 for the latter. Thus, a simple, accurate amidolytic assay and a radioimmunoassay have been devised for measuring factor XI in plasma that correlate well with the coagulant activity of factor XI, as determined in our laboratory.

Scott, C. F., Sinha, D., Seaman, F. S., *Walsh, P. N.*, and Colman, R. W.

Blood 63(1):42-50, 1984.

Other support: National Institutes of Health.

From the Thrombosis Research Center and Hematology-Oncology Section, Department of Medicine, Temple University School of Medicine, Philadelphia.

INTERACTION OF COAGULATION PROTEINS WITH PLATELETS: POSSIBLE SIGNIFICANCE FOR MAINTENANCE OF HEMOSTASIS AND PATHOGENESIS OF THROMBOEMBOLISM

The purpose of this chapter is to present some of the evidence that platelets possess binding sites for a variety of coagulation proteins and that these specific platelet coagulant protein interactions can promote zymogen activations at various stages of intrinsic coagulations including the initiation of intrinsic coagulation. In addition, studies are presented that are consistent with the hypothesis that these interactions of coagulation proteins with platelets are important physiologically in promoting normal hemostasis and pathologically in predisposing to certain venous and arterial thrombotic diseases.

Walsh, P. N.

In: *Atherosclerosis Reviews* 12, Raven Press, NY, 1984, pp 129-143.

Other support: U. S. Department of Health, Education and Welfare.

From the Thrombosis Research Institute, Department of Medicine, Temple University School of Medicine, Philadelphia.

BLOOD COAGULATION FACTOR XIa BINDS SPECIFICALLY TO A SITE ON ACTIVATED HUMAN PLATELETS DISTINCT FROM THAT FOR FACTOR XI

Binding of ^{125}I-Factor XIa to platelets required the presence of high molecular weight kininogen, was enhanced when platelets were stimulated with thrombin, and reached a plateau after 4-6 min of incubation at 37C. Factor XIa binding was specific: 50- to 100-fold molar excesses of unlabeled Factor XIa prevented binding, whereas Factor XI, prekallikrein, Factor XIIa, and prothrombin did not. When washed erythrocytes, added at concentrations calculated to provide an equivalent surface area to platelets, were incubated with Factor XIa, only a low level of nonspecific, nonsaturable binding was detected. Factor XIa binding to platelets was partially reversible and was saturable at concentrations of added Factor XIa of 0.2-0.4 μg/ml (1.25-2.5 μM). The number of Factor XIa binding sites on activated platelets was estimated to be 225 per platelet (range 110-450). We conclude that specific, high affinity, saturable binding sites for Factor XIa are present on activated platelets, are distinct from those previously demonstrated for Factor XI, and require the presence of high molecular weight kininogen.

Sinha, D., Seaman, F. ., Koshy, A., Knight, L. C., and *Walsh, P. N.*

Journal of Clinical Investigation 73:1550-1556, 1984.

Other support: National Institutes of Health and American Heart Association, Pennsylvania Affiliate.

From the Thrombosis Research Center, Section of Hematology/Oncology, Department of Medicine, Temple University School of Medicine, Philadelphia.

FACTOR V: A PLATELET CYTOSKELETAL ASSOCIATED PROTEIN

Platelet cytoskeletons prepared from thrombin activated platelets contain specifically associated Factor Va. We postulate that Factor Va associates with the cytoskeleton through receptors present on the platelet surface. The following evidence supports this hypothesis: (a) Prior secretion of Factor Va is necessary for the association of Factor Va on the cytoskeleton. (b) Reagents that inactivate Factor Va on the surface of the platelet, such as EDTA and proteolytic enzymes, also inactivate the Factor Va on the cytoskeleton. (c) The platelet Factor Xa binding site(s) (Factor Va) are quantitatively retained on the platelet cytoskeleton. (d) Platelet cytoskeletons prepared from platelets that are thought to be deficient in Factor Va binding sites contain 25% of Factor Va activity of normal platelet cytoskeletons and platelet cytoskeletons prepared from platelets deficient in Factor Va contain no Factor Va activity but regain control levels of Factor Va only when Factor Va is added to the platelets prior to the preparation of cytoskeletons.

Tuszynski, G. P. and *Walsh, P. N.*

Haematologia 17(1):67-76, 1984.

Other support: U. S. Department of Health and Human Services.

From the Thrombosis Research Center, Temple University School of Medicine, Philadelphia.

THE PLATELET CYTOSKELETON CONTAINS ELEMENTS OF THE PROTHROMBINASE COMPLEX

Triton-insoluble cytoskeletons prepared from thrombin-activated platelets were found to potentiate the activation of prothrombin (prothrombinase activity). Cytoskeletons prepared from red cells or lymphoblasts contained no prothrombinase activity. The platelet prothrombinase activity was dependent on cytoskeletal-associated Factor Va, and exogenously added Factor Xa and prothrombin. Cytoskeletons contained 38% of the total platelet prothrombinase activity. Both platelets and cytoskeletons displayed half-maximal activities at similar prothrombin concentrations. The role of lipids in the cytoskeletal prothrombinase activity was investigated. Cytoskeletons were found to contain 3.8% of the total platelet phospholipids, consisting of the following lipids expressed as percentage of total present in platelets: 6.0% sphingomyelin, 3.8% phosphatidylcholine, 2.9% phosphatidyl-ethanolamine, 4.4% phosphatidylinositol, and 2.2% phosphatidylserine. The cytoskeletal prothrombinase activity and the lipid phosphorus content of cytoskeletons decreased after treatment of cytoskeletons with various doses of phospholipase C. Incubation of cytoskeletons with the highest concentrations tested (10 μg/ml) resulted in a 72% loss of phosphatidylserine and 84% loss of cytoskeletal prothrombinase activity. Cytoskeletal prothrombinase activity destroyed by phospholipase C treatment could be restored to control levels by treatment of hydrolyzed cytoskeletons with total cytoskeletal lipid or mixtures of phosphatidylserine/phosphatidylcholine (25:75% by weight). These results suggest that the cytoskeletal prothrombinase complex in addition to containing Factor Va, as has been previously shown, contains a lipid cofactor activity consisting in part of phosphatidylserine.

Tuszynski, G. P., Maucos, G. P., Koshy, A., Schick, P. K., and *Walsh, P. N.*

The Journal of Biological Chemistry 259(11):6947-6951, 1984.

Other support: U. S. Department of Health and Human Services.

From the Thrombosis Research Center, Temple University School of Medicine, Philadelphia.

COMPARISON OF BLEEDING TENDENCY, FACTOR XI COAGULANT ACTIVITY, AND FACTOR XI ANTIGEN IN 25 FACTOR XI-DEFICIENT KINDREDS

The relationship of clinical bleeding tendency and factor XI antigen (XI:Ag) in factor XI deficiency was studied in 78 members of 25 factor XI-deficient kindreds. Factor XI:Ag was measured in a competitive radioimmunoassay using monospecific, heterologous anti-factor XI antibody, ^{125}I-labeled factor XI, and staphylococcal protein A as the precipitating agent. Deficiency of factor XI clotting activity (XI:C), < 0.62 U/ml occurred in 48 individuals, 22 of whom experienced postoperative or posttraumatic bleeding. Their mean factor XI:C was 0.21 ± 0.04 U/ml (SEM) and factor XI:Ag was

0.23 ± 0.04 U/ml. The remaining 26 had no clinical bleeding, many despite surgical challenge. Their mean factor XI:C was 0.30 ± 0.04 U/ml and factor XI:Ag was 0.34 ± 0.05 U/ml. In all, 13 kindreds had between 1 and 11 members with bleeding; the other 12 had none with deficient hemostasis. Two heterozygous factor XI-deficient individuals appeared to be positive for cross-reacting material (CRM). The slope of the regression line for factor XI:C and factor XI:Ag data points in the 78 individuals tested did not differ from control and all points fell within 95% confidence limits derived from control. In conclusion, bleeding tendency appears to be consistent within a given kindred and is not determined exclusively by factor XI:C or factor XI:Ag levels.

Ragni, M. V., Sinha, D., Seaman, F., Lewis, J. H., Spero, J. A. and *Walsh, P. N.*

Blood 65(3):719-724, 1985.

Other support: National Institutes of Health.

From the Department of Medicine, University of Pittsburgh; Central Blood Bank, Pittsburgh; Thrombosis Research Center, Temple University School of Medicine, Philadelphia.

FUNCTIONAL CHARACTERIZATION OF HUMAN BLOOD COAGULATION FACTOR XIa USING HYBRIDOMA ANTIBODIES.

During the initiation of intrinsic coagulation, factors XI and XIa interact intimately with several other coagulation proteins (factor XIIa, high M, kininogen, and factor IX) as well as with the platelet surface. To help elucidate these complex intramolecular interactions, we have prepared a collection of monoclonal antibodies directed against various epitopes in Factor XI. We have utilized these reagents to isolate factor XI and the light chain of factor XIa on affinity columns and to probe structure-function relationships involved in the interactions of factor XIa with factor IX. The isolated light chain of factor XIa retained > 90% of its amidolytic activity against the oligopeptide substrate pyro-Glu-Pro-Arg-pNA (S-2366), but only 3.8% of its clotting activity in a factor XIa assay and 1% of its factor IX activating activity in an activation peptide release assay. This suggests that regions of the heavy chain are required for development of coagulant activity and specifically for the interaction of factor XIa with factor IX. To test this hypothesis, the effects of three of the monoclonal antibodies (5F4, 1F1, and 3C1) on the function of factor XIa were examined. The results show that in a clotting assay the light chain-specific antibody (5F4) inhibits 100% of the factor XIa activity, whereas of the heavy chain-specific antibodies, one (3C1) inhibits 75% and another (1F1) only 17%. Similarly, in the factor IX activation peptide release assay antibody 5F4 inhibits 100% of the factor XIa activity, whereas 3C1 inhibits 75% and 1F1 inhibits 33%. We conclude that regions located in the heavy chain, in addition to those in the light chain, are involved in the interaction of factor XIa with factor IX and in the expression of the coagulant activity of factor XI.

Sinha, D., Koshy, A., Seaman F. S., and *Walsh, P. N.*

The Journal of Biological Chemistry 260(19):10714-10719, 1985.

Other support: U. S. Department of Health and Human Services, W.W. Smith Charitable Trust, American Heart Association Biomedical Research, and Division of Research Resources, National Institutes of Health.

From the Thrombosis Research Center, Department of Medicine, Temple University School of Medicine, Philadelphia, PA.

PLATELET-MEDIATED COAGULANT PROTEIN INTERACTIONS IN HEMOSTASIS

From the evidence reviewed here, it is possible to construct an overview of the hemostatic mechanism emphasizing the essential contributions of platelets in zymogen activations at each stage of intrinsic coagulation. Specific high-affinity receptors for various coagulation proteins are exposed on the platelet surface membrane. The coagulation proteins so far shown to bind the platelet membrane include the factor XI-high-mol wt kininogen complex, factor XIa, the factor Xa-factor Va complex, thrombin, and fibrinogen. As a consequence of these interactions, platelets promote the activation of prekallikrein, factor XII, factor XI, factor IX, factor X, and prothrombin. Platelets also appear to participate in a variety of alternative reaction mechanisms that may minimize or obviate the requirement for certain coagulation proteins, including factor XII and factor XI. This function of platelets may account for the absence of hemostatic defects in patients with deficiencies of some of the proteins involved in the contact phase of intrinsic coagulation. Finally, platelets appear to protect certain coagulation proteins, such as factor XIa and factor Xa, from inactivation by plasma proteinase inhibitors. These various functions of platelets are seen as localizing coagulation reactions to the hemostatic plug that occurs at loci of blood vessel injury.

Walsh, P. N.

Seminars in Hematology, 22(3): 176-186, 1985.

Other support: National Institutes of Health and American Heart Association.

From the Thrombosis Research Center, Temple University School of Medicine, Philadelphia.

POTENTIATION OF THE ACTIVITY OF COAGULATION FACTOR XI BY HUMAN PLATELETS

The present studies demonstrate that when washed platelets or platelet membranes are incubated with purified factor XI and the mixtures are assayed for factor XI activity, a 20-fold potentiation of coagulant activity is observed. This synergism is dependent on the presence of active factor XI and intact platelet membranes since it is abolished either by preincubation of factor XI with monospecific anti-factor XI antibody, or by preincubation of platelet membranes with phospholipase C. The saturable nature of the potentiation of purified factor XI by platelet membranes suggests the possibility that it represents a functional correlate of the saturable, specific binding of factor XI to platelets previously demonstrated. This suggestion is supported by the demonstration that platelets enhance the rate of proteolytic activation of factor XI in the presence of either factor XIIa or kallikrein.

Walsh, P. N. and Tuszynski, G. P.

Thrombosis Research 40:257-266, 1985.

Other support: National Institutes of Health, American Heart Association and W.W. Smith Charitable Trust.

From the Thrombosis Research Center, Department of Medicine, Temple University School of Medicine, Philadelphia.

EFFECT OF PROSTANOIDS ON THE CORONARY CIRCULATION

An accurate evaluation of the physiological and pathophysiological role of endogenous prostanoids in the human coronary circulation has to rely upon the joint results from studies using different techniques. In the present paper some data obtained in this laboratory are presented, which are aimed to elucidate the physiological and pathophysiological involvement of endogenous prostanoids in the regulation of coronary blood flow. Furthermore, some data on the pharmacology of PGI_2 in patients with acute myocardial infarction (AMI) are included. Based on the data presented here and on the considerations discussed it seems obvious that coronary prostanoid formation is limited both in healthy subjects and in patients with IHD. This is evidenced both by the lack of detectable release of PGI_2, the main coronary prostanoid formed, under normal conditions and under physiological stimulation of coronary blood flow, as well as under conditions of manifest cardiac ischaemia. On the other hand, the lack of significant release of PGI_2 is not due to a limited capacity to such formation, as evident from the marked release of 6-keto-$PGF_{1\alpha}$ in patients subjected to cardioplegia. Although the data presented here do not support the concept of a significant role for coronary prostanoids in health and disease the results presented on interaction between adenosine and prostanoids in the regulation of forearm blood flow may be of significance also in the coronary circulation, implying that prostanoids may play the role of a vasodilator messenger which transfers the signal to vascular relaxation from the endothelium to the vascular smooth muscle.

Wennmalm, A. et al.:

In: *IUPHAR 9th Int. Cong. of Pharmacol. London*, 1984. Proceedings, vol. 3, pp. 13-19, Macmillan Press, London, 1984.

Other support: The Swedish Medical Research Council and the National Research Council of Italy.

From the Department of Clinical Physiology, Karolinska Institute, Huddinge University Hospital, and Karolinska Hospital, Stockholm, Sweden; and Department of Pharmacology, Catholic University, Rome, Italy.

EFFECTS OF CHRONIC EXPOSURE TO CIGARETTE SMOKE ON AMINE LEVELS AND TURNOVER IN VARIOUS HYPOTHALAMIC CATECHOLAMINE NERVE TERMINAL SYSTEMS AND ON THE SECRETION OF PITUITARY HORMONES IN THE MALE RAT

Male rats were exposed to the smoke from 2 cigarettes every morning for a total period of 9 days. The next day they were decapitated immediately after the exposure to the smoke from 4 cigarettes (Kentucky reference 1R-1 type) burned at 30-min intervals. Control animals were exposed to air alone or to nicotine-free cigarette smoke (Cambridge glass fiber filters). In contrast to chronic exposure to filtered smoke, exposure to unfiltered smoke resulted in a 10% increase in catecholamine (CA) levels (quantitative histofluorimetry) within the lateral palisade zone, the posterior periventricular hypothalamic nucleus and within the dorsomedial hypothalamic nucleus. There was also an increase in amine turnover (tyrosine hydroxylase inhibition by α-methyl-*dl*-*p*-tyrosine methylester (αMT) in the dopamine (DA) systems of the medial and lateral palisade zones and in the periventricular noradrenaline (NA) hypothalamic systems. Chronic exposure to unfiltered cigarette smoke resulted in reductions of prolactin, LH and FSH levels (radioimmunoassay). Following αMT treatment, chronic exposure to unfiltered cigarette smoke still led to reduced prolactin serum levels. In addition, an increased vasopressin serum concentration was found. The effects of chronic exposure to cigarette smoke on neuroendocrine function and on hypothalamic CA systems are suggested to be mediated via nicotine. Combined with the results from a previous study, the present results indicate that tolerance does not develop with regard to the inhibitory effects of exposure to cigarette smoke on prolactin, LH and FSH secretions. The same is true for the stimulatory effects on the tubero-in-fundibular DA neurons and the periventricular NA systems. But chronic exposure to cigarette smoke seemed to induce tolerance with regard to its stimulatory effects on subependymal dorsomedial and paraventricular hypothalamic NA systems and on corticosteronesone release.

Andersson, K., Eneroth, P., *Fuxe, K.*, Mascagni, F., and Agnati, L. F.

Neuroendocrinology 41(6):462-466, 1985.

From the Department of Histology, Karolinska Institute, and Research and Development Laboratory, Department of Obstetrics and Gynecology, Karolinska Hospital, Stockholm; Department of Human Physiology and Endocrinology, University of Modena, Modena, Italy.

EFFECTS OF ACUTE CENTRAL AND PERIPHERAL ADMINISTRATION OF NICOTINE ON ASCENDING DOPAMINE PATHWAYS IN THE MALE RAT BRAIN. EVIDENCE FOR NICOTINE INDUCED INCREASES OF DOPAMINE TURNOVER IN VARIOUS TELENCEPHALIC DOPAMINE NERVE TERMINAL SYSTEMS.

The actions of intraventricular injections and intravenous infusions of nicotine were studied on dopamine stores and turnover in discrete areas of the forebrain of normal male rats. This was done by measuring the decline of the dopamine stores after tyrosine hydroxylase inhibition using α-methyl-tyrosine methyl ester (H 44/68). The dopamine concentrations in the various telencephalic dopamine nerve terminal systems were measured using the Falck-Hillarp methodology involving quantitative

microfluorimetry. The catecholamine concentrations in the anteromedial frontal cortex were measured biochemically, using high pressure liquid chromatography combined with electrochemical detection.

Intraventricular experiments. The dopamine levels in discrete areas of nuc. caudatus and nuc. accumbens were significantly reduced even with the lowest dose of nicotine (1 μg/rat). Intraventricular injections of nicotine in a dose of 100 μg/rat produced significant increases of dopamine turnover in various types of dopamine nerve terminal systems in the nuc. caudatus, nuc. accumbens, and tuberculum olfactorium, and following a dose of 10 μg/rat, increases of dopamine turnover were observed in the medial part of the nuc. caudatus. Furthermore, nicotine (100 μg/rat) significantly increased noradrenaline but not dopamine turnover with the anterofrontal cortex.

Intravenous experiments. The dopamine levels were selectively reduced by nicotine (1000 μg/kg) in the cholecystokinin positive and negative dopamine nerve terminal systems of the nuc. accumbens. On the other hand, dopamine levels in the anteromedial frontal cortex were increased after this dose of nicotine. Intravenous infusions of nicotine (10-1000 μg/kg) produced dose-related increases of dopamine turnover in the various dopamine nerve terminal systems analyzed in the telencephalon. These effects became significant with a dose of 1000 μg/kg/h. The dopamine terminals in the nuc. caudatus showed a higher sensitivity to intravenous infusions of nicotine, being affected by 10-100 μg/kg of nicotine.

These findings suggest that relatively low doses of nicotine via an activation of central nicotine-like cholinergic receptors can reduce dopamine concentration and increase dopamine turnover in discrete limbic and striatal areas. These actions may in part represent the neurochemical basis for the rewarding actions of nicotine and for nicotine dependence in man.

Andersson, K., *Fuxe, K.*, Agnati, L. F., and Eneroth, P.

Medical Biology 59:170-176, 1981.

Other support: Swedish Tobacco Company.

From the Department of Histology, Karolinska Institute, Stockholm, Sweden, and the University of Modena, Modena, Italy.

EFFECT OF MATERNAL NICOTINE ON THE DEVELOPMENT OF SITES FOR [^{3}H]NICOTINE BINDING IN THE FETAL BRAIN

The sites for [^{3}H]nicotine binding in fetal brains were examined after administration of nicotine into pregnant rats. Administration of unlabeled nicotine into the pregnant rats increased B_{max} values for the sites for tririared nicotine binding without affecting K_d values in the fetal brains. Treatment with this regimen, however, did not show any significant change in the sites for [^{3}H]quinuclidinyl benzylate binding. In addition, treatment with this regimen increased B_{max} values of the sites for [^{3}H]nicotine binding in the brains of pregnant rats. α-Bungarotoxin had no effect on the sites for [^{3}H]nicotine binding. It is inferred, therefore, that a similar response is elicited by nicotine binding sites to administered nicotine in both the fetal and maternal brains. Furthermore, a possible effect of nicotine in pregnant rats may be the facilitation of the development of nicotine acetylcholine receptors in the fetal brain.

Hagino, N. and Lee, J. W.

International Journal of Developmental Neuroscience 3(5):567-571, 1985.

From the Department of Cellular and Structural Biology, The University of Texas Health Science Center at San Antonio.

THE ROLE OF MATERNAL NICOTINE IN THE DEVELOPMENT OF NICOTINIC ACETYLCHOLINE RECEPTORS AND HYPOTHALAMIC DOPAMINERGIC NEURONS IN THE FETAL BRAIN

It has already been reported that delayed development of hypothalamic dopaminergic (TIDA) neurons in the fetal brain caused a delay of differentiation of prolactin cells and puberty onset in offspring of rats. From observations mentioned, it seems likely that maternal neurotransmitters and/or hormones could influence the development of TIDA neurons and differentiation of prolactin cells in offspring. Preliminary results indicate that maternal and placental acetylcholine (Ach) seem to act on nicotinic sites to activate the nicotinic acetylcholine receptors (nAchR) and influence the development of TIDA neurons in the fetal brain. This study tends to lend support to a working hypothesis that nAchRs appear in the fetal brain during an earlier stage of gestation. During the last week of gestation, maternal and placental Ach act on nicotinic sites to activate Ca^{2+} flux which stimulates TIDA neurons through activation of protein kinase and phosphorylation. Thus, maternal nicotine could influence the growth and development of TIDA neurons in the fetal brain.

Hagino, N.

In: Caciagli, F., Giacobini, E., and Paoletti, R. (eds.) *Developmental Neuroscience: Physiological, Pharmacological and Clinical Aspects*, New York: Elsevier Science Publishers B. V., 1984, pp. 127-130.

From the Department of Cellular and Structural Biology, The University of Texas Health Science Center, San Antonio.

[^{3}H]NICOTINE BINDING SITES IN DEVELOPING FETAL BRAINS IN RATS

[^{3}H]Nicotine binding sites were examined in developing fetal brains in rats. The fetal brain membranes bound [^{3}H]nicotine with an affinity similar to that of adult brain membranes. This binding was displaced by unlabeled nicotine or carbamylcholine, the inhibition concentrations being approximately the same for fetal and adult brain preparations. α-Bungarotoxin had no effect in [^{3}H]nicotine binding to fetal brains membranes as well as to adult brain preparations. The specific [^{3}H]nicotine binding was first detectable on day 16 of gestation and increased several fold until birth.

Sugiyama, H., *Hagino, N.*, Moore, G., and Lee, J. W.

Neuroscience Research 2:387-392, 1985.

From the Department of Cellular and Structural Biology, The University of Texas Health Science Center at San Antonio, and the Department of Cellular Physiology, National Institute of Physiological Sciences, Okazaki, Japan.

GUANINE NUCLEOTIDES REGULATE [^{3}H]SUBSTANCE P BINDING IN RAT SMALL INTESTINE

The binding of [^{3}H]substance P (SP) to membranes of the rat small intestine demonstrates specific binding to receptors having more than one affinity for SP. The values of the binding parameters for the high-affinity site obtained from a non-linear

regression analysis are as follows: K_D = 0.25 nM, B_{max} = 149.5 fmol/mg protein. Inhibition curves of ^{3}H-SP binding using various unlabeled tachykinins show that the high-affinity receptor is of the P-subtype, having the highest affinity for SP and lower affinities for eledoisin and kassinin. Guanine nucleotides and sodium independently reduce the binding of ^{3}H-SP to the high-affinity receptor in a dose-related manner; GTP and GDP are more potent than GMP. The reduction of specific SP binding by GTP can be ascribed primarily to an increase in the off-rate. The effects of guanine nucleotides on ^{3}H-SP binding to membranes of rat small intestine suggest that the high-affinity receptor is linked to an effector by a GTP-binding regulatory protein.

Smith, K.E. and *Hoss, W.P.*

Regulatory Peptides 11:275-285, 1985.

Other support: National Institutes of Health.

From the Center for Brain Research, University of Rochester, School of Medicine and Dentistry, Rochester, NY.

CHARACTERIZATION OF MUSCARINIC CHOLINERGIC RECEPTORS IN THE BRAINS OF COPPER-DEFICIENT RATS

In order to assess a possible role for copper as a regulator of muscarinic receptors *in vitro*, the receptor was characterized in rats made copper deficient by a dietary regimen. In forebrain regions there was a decrease in both the affinity of the receptors for [^{3}H]-1-quinuclidinyl benzilate and the density of receptors in the copper-deficient animals compared with control animals. Copper treatment *in vitro* of homogenates from deficient animals did not reverse the *in vivo* effects on antagonist binding but, rather, decreased receptor occupancy and ligand affinity in a manner similar to copper treatment of control homogenates. Minimally deficient rats displayed very similar changes in receptor properties compared with the more severely deficient animals. Minimal copper deficiency produced robust effects on the binding of agonists, increasing ID_{50} and derived dissociation constants. The addition of copper to the assay medium caused an apparent reversal of the *in vivo* effect of copper deficiency on agonist binding, decreasing ID_{50} and derived dissociation constants to values near those observed with homogenates from normal animals in the presence of copper. Since copper deficiency has dramatic effects on both receptor number and the binding of agonists to muscarinic receptors in the central nervous system, it is suggested that copper, because of its ability to form complexes with some proteins, may have an endogenous role in the regulation of the receptor.

Farrar, J. R., *Hoss, W.*, Herndon, R. M., and Kuzmiak, M.

The Journal of Neuroscience 5(4):1083-1089, 1985.

Other support: National Institutes of Health.

From the Center for Brain Research, University of Rochester School of Medicine and Dentistry, Rochester, NY.

PROTEIN PHOSPHORYLATION AND NEURONAL FUNCTION

Studies in the past several years have provided direct evidence that protein phosphorylation is involved in the regulation of neuronal function. Electro-

physiological experiments have demonstrated that three distinct classes of protein kinases, *i.e.*, cyclic AMP-dependent protein kinase, protein kinase C, and CaM kinase II, modulate physiological processes in neurons. Cyclic AMP-dependent protein kinase and kinase C have been shown to modify potassium and calcium channels, and CaM kinase II has been shown to enhance neurotransmitter release. A large number of substrates for these protein kinases have been found in neurons. In some cases (*e.g.*, tyrosine hydroxylase, acetylcholine receptor, sodium channel) these proteins have a known function, whereas most of them (*e.g.*, synapsin I) had no known function when they were first identified as phosphoproteins. In the case of synapsin I, evidence now suggests that it regulates neurotransmitter release. These studies of synapsin I suggest that the characterization of previously unknown neuronal phosphoproteins will lead to the elucidation of previously unknown regulatory processes in neurons.

Browning, M. D., *Huganir, R.*, and Greengard, P.

Journal of Neurochemistry 45(1):11-23, 1985.

Other support: National Science Foundation, U. S. Public Health Service, and a contract from the U. S. Air Force School of Aerospace Medicine.

From the Laboratory of Molecular and Cellular Neuroscience, The Rockefeller University, New York.

EFFECT OF NICOTINE ON CEREBRAL PROTEIN METABOLISM DURING DEVELOPMENT

Several studies on the effects of nicotine on fetal growth have suggested that altered protein synthesis is the underlying mechanism of changes in gestational length, brain weight, or behavior. Discrepancies were found in gross structural changes; in some instances fetal size and brain weights were not found to be different after exposure to nicotine. However, most results indicate behavioral changes in the offspring. The results presented here suggest that nicotine does affect cerebral protein metabolism in both developing and adult brain. These researchers examined the rates of protein metabolism in the offspring of rats administered nicotine during gestation to assess whether changes in weight or protein content occur and whether they are related to alterations of protein metabolism. Results showed that there were no gross morphological changes in the offspring of rats administered nicotine during gestation. Cortical and cerebellar brain weights were similar to control values at 2 and 4 days after birth. Total protein content was also similar. However, measured synthesis rates were 20% and 5% lower in cortex of nicotine-treated animals at 2 and 4 days after birth, respectively. Calculated degradation rates (percent synthesis per h minus percent protein increase per h) are therefore lower in the nicotine animals to account for the lack of change in protein in these animals. Therefore, on the basis of this study, it appears that nicotine influences brain function, and that protein degradation and synthesis processes in fetal-newborn rat brain are affected by nicotine.

Sershen, H., Reith, M. E. A., and *Lajtha, A.*

In: Caciagli, F., Giacobini, E., and Paoletti, R. (eds.) *Developmental Neuroscience: Physiological, Pharmacological and Clinical Aspects*, New York: Elsevier Science Publishers B. V., 1984, pp. 119-122.

From the Center for Neurochemistry, Nathan S. Kline Institute for Psychiatric Research, Ward's Island, New York.

NICOTINE BINDING SITES IN BRAIN

In the past 10 years, many attempts have been made to identify sites in brain that bind labeled nicotine. However, in these studies no estimation was made of saturable versus non-saturable binding. More recent studies show curvilinear Scatchard plots, suggesting the presence of multiple sites. Nicotinic or muscarinic antagonists have little or no affinity to the binding site, which has been suggested by Romano and Goldstein to be due to an agonist-induced shift of the receptor to a high-affinity agonist selective state. The kinetics of binding indicate a high-affinity component with a K_d in the range of 0.1-60 nM, and a low-affinity component with affinities that vary widely from one preparation to another. The present investigators have compared the binding of (±)-[^{3}H] nicotine and [^{3}H] acetylcholine in various brain regions Although midbrain showed the highest and cerebellum the lowest binding for both nicotine and acetylcholine, the ratio of nicotine/acetylcholine binding showed a three-fold regional variation. Although there is still much variability in the results of binding studies from various laboratories, nicotine binding in brain is probably not solely related to the classical nicotine receptors. Multiple binding sites have been reported, and may be related to the multiple effects of nicotine on the central nervous system.

Sershen, H., Reith, M. E. A., and *Lajtha, A.*

In: Caciaglia, F., Giacobini, E., and Paoletti, R. (eds.) Developmental Neuroscience: Physiogical, Pharmacological and Clinical Aspects, New York: Elsevier Science Publishers B.V., 1984, pp. 147-150.

From the Center for Neurochemistry, Nathan S. Kline Institute for Psychiatric Research, Ward's Island, New York.

FREE RADICAL MEDIATED ALTERATIONS OF THE SYNAPSE

Previous work from this laboratory demonstrated that in the frog cutaneous pectoris neuromuscular junction, synaptic vesicles can be induced to reversibly fuse with the plasma membrane of the nerve terminal by the application of a Ringer's solution containing 105 mM K^+-propionate and that the exposure of this externalized vesicular membrane to horseradish peroxide (HRP) leads to vesicular membrane alterations during recycling. Similar HRP exposure of the synaptic vesicle membrane of 0.1 mm cerebral cortex slices results in both the reduction of high-affinity [^{14}C]-gamma-aminobutyric acid (GABA) uptake and in easily distinguishable vesicular membrane alterations after recycling. Brain slices initially incubated for 60 minutes in depolarizing (K^+) buffer containing HRP, followed by a 60-minute incubation in HEPES (synaptic vesicle recovery), exhibit a 30% reduction of [^{14}C]GABA uptake and the appearance of nonsynaptic vesicle membrane in the nerve terminal. The combination of these morphological and biochemical data points to the existence of a relationship between high-affinity neurotransmitter uptake and synaptic vesicle membrane. The fact that non-K^+-stimulated cerebral cortex slices (no synaptic vesicle-plasma membrane fusion) which are exposed to HRP (in HEPES) for 60 minutes exhibit no reduction of [^{14}C]GABA uptake indicates that fused vesicle membrane is susceptible to HRP damage and that damage to these membranes alters the neurotransmitter transport system. In order to isolate and better study the functional synaptic unit, synaptosomes (cerebral cortex) were prepared and exposed to HRP. In the absence of K^+-depolarization, synaptosomes exposed to HRP for 45 minutes take up 70% less [^{14}C]GABA. Observation of this HRP-induced reduction of synaptosomal neurotransmitter uptake in relation to the

HRP-induced synaptic vesicle membrane alterations observed in mouse cerebral cortex slices indicates that the plasma membranes of these isolated synaptosomal units are themselves susceptible to HRP-induced damage and that the damage of this membrane also alters neurotransmitter uptake.

Debler, E. A., Sershen, H., *Lajtha, A.*, and Gennaro, J. F., Jr.

Annals of the New York Academy of Sciences 435:140-144, 1984.

From the Department of Biology, New York University, and the Center for Neurochemistry, Ward's Island, New York.

ENDOGENOUS MATERIAL IN BRAIN INHIBITING [^{3}H]NICOTINE AND [^{3}H]ACETYLCHOLINE BINDING

The supernatant obtained from mouse brain homogenates contains material that inhibits the saturable binding of [^{3}H]nicotine in mouse cerebral cortex. This inhibitory material was further purified by heat denaturation, ultrafiltration through an Amicon PM-10 membrane filter, and gel chromatography on Sephadex G-10. The material inhibited the binding of [^{3}H]acetylcholine with the same potency as it did that of [^{3}H]nicotine. It also had some affinity for the sites that specifically bind [^{3}H]D-Ala, D-Leu enkephalin, but had much lower affinity for the binding sites for tritiated quinuclidinyl benzilate (QNB), spiroperidol, naloxone, or imipramine. Acid hydrolysis destroyed the activity. These preliminary results suggest the presence in brain of "nicotine-like" substances, one of which may be the endogenous ligand for the sites that specifically bind [^{3}H]nicotine.

Sershen, H., Reith, M. E. A., Hashim, A., and *Lajtha, A.*

Journal of Neuroscience Research 12(4):563-569, 1984.

Other support: New York State Health Research Council.

From the Center for Neurochemistry, Nathan S. Kline Institute for Psychiatric Research, Ward's Island, New York.

COMPARISON OF [^{3}H]NICOTINE AND [^{3}H]ACETYLCHOLINE BINDING IN MOUSE BRAIN: REGIONAL DISTRIBUTION

In a continuing study of nicotine binding sites, the authors determined the relative amount of nicotine binding and acetylcholine binding in various brain regions of C57/BL and of DBA mice. Although midbrain showed the highest and cerebellum the lowest binding for both [^{3}H]nicotine and [^{3}H]acetylcholine, the ratio of nicotine to acetylcholine binding showed a three-fold regional variation. Acetylcholine inhibition of [^{3}H]nicotine binding indicated that a portion of nicotine binding was not inhibited by acetylcholine. These results indicate important differences between the binding of (±)-[^{3}H]nicotine and that of [^{3}H]acetylcholine.

Sershen, H., Reith, M. E. A., Hashim, A., and *Lajtha, A.*

Research Communications in Chemical Pathology and Pharmacology 48(3):145-352, 1985.

From the Center for Neurochemistry, Nathan S. Kline Institute for Psychiatric Research, Ward's Island, New York.

LOCAL AND SYSTEMIC CAPSAICIN PRETREATMENT INHIBITS SNEEZING AND THE INCREASE IN NASAL VASCULAR PERMEABILITY INDUCED BY CERTAIN CHEMICAL IRRITANTS

1. The effects of local exposure to chemical irritants and mechanical stimulation on sneezing reflexes have been studied in normal and capsaicin-pretreated, conscious guinea-pigs. The influence of local and systemic capsaicin pretreatment on vascular permeability to plasma proteins and the cardiovascular effects of local application of capsaicin to the nasal mucosa have also been studied in anaesthetized animals.

2. Local application of capsaicin (threshold dose 3 μM), nicotine (threshold dose 300 μM) or formalin to the nasal mucosa induced reflex sneezing discharges. Systemic or local capsaicin pretreatment abolished or reduced the sneezing responses to capsaicin and formalin. The response to nicotine was also reduced following local pretreatment with capsaicin, while the response to systemic pretreatment with capsaicin was only slightly affected. The sneezing response to mechanical stimulation was not affected by capsaicin pretreatment.

3. Pretreatment with a local anaesthetic induced a similar dose-dependent inhibition of the sneezing responses to both capsaicin and nicotine.

4. Local application of disodium cromoglycate to the nasal mucosa reduced the sneezing response to capsaicin, but not that to nicotine.

5. Local pretreatment with the 3 mM and 30 mM capsaicin solution inhibited the increase in vascular permeability to plasma proteins in the nasal mucosa induced by i.v. capsaicin. Local pretreatment with capsaicin did not result in any reduction in the capsaicin-induced permeability in the ureter, suggesting that such treatment did not have any major systemic toxic effects.

However, a small, acute increase in respiratory insufflation pressure, indicating bronchoconstriction, was seen when the 30mM capsaicin solution was applied to the nasal mucosa. The application of capsaicin (3 mM and 30 mM) to the nasal mucosa resulted in an increase in arterial blood pressure and tachycardia due to reflex sympathetic activation.

6. Exposure of normal guinea-pigs to an atmosphere saturated with ether caused excited avoidance behaviour and intense nose wipings with the fore paws. This response was abolished by systemic pretreatment with capsaicin and reduced by local capsaicin pretreatment.

7. Local application of serotonin, histamine, leukotriene C_4, bradykinin, phenyldiguanide, substance P (SP) or [d-Arg, d-Pro, d-Trp, Leu]SP, and SP-antagonist, did not induce any sneezing. High concentrations of compound 48/80 caused a small sneezing response. Local pretreatment with the SP-antagonist (7×10^{-4}M) did not influence the sneezing responses to nicotine or capsaicin.

8. It is concluded that only substances that are known to activate sensory nerves induce sneezing. Furthermore, there seems to be at least two types of afferent nerves in the nasal mucosa which respond to specific chemical irritation. One type, capsaicin-sensitive nerves, which respond to capsaicin, formalin, ether and nicotine, while another type of afferent nerves involved in sneezing reflexes is largely resistant to capsaicin pretreatment and is activated by nicotine. Local application of capsaicin to the nasal mucosa may thus be a selective way of reducing nasal reactivity to certain chemical irritants without causing systemic degeneration of capsaicin-sensitive C-fiber afferents.

Lundblad, L., *Lundberg, J. M.*, and Anggard, A.

Archives of Pharmacology 326:254-261, 1984.

Other support: Swedish Medical Research Council; the Swedish Tobacco Company; the Astra Foundation; the Swedish Society of Medical Science; and the Funds of the Karolinska Institute.

From the Department of Oto-Rhino-Laryngology, Karolinska Hospital, and the Department of Pharmacology, Karolinska Institute, Stockholm, Sweden.

EFFECTS OF NEUROPEPTIDE Y (NPY) ON MECHANICAL ACTIVITY AND NEUROTRANSMISSION IN THE HEART, VAS DEFERENS AND URINARY BLADDER OF THE GUINEA-PIG

The effects of preincubation for 10 min with synthetic porcine neuropeptide Y (NPY) on muscle tone and autonomic transmission in the guinea-pig right atrium, vas deferens, urinary bladder, portal vein and trachea were analysed in vitro. NPY induced a metoprolol-resistant, long-lasting, positive inotropic and cronotropic effect *per se* in the spontaneously beating right atrium. Furthermore, NPY caused a reversible inhibition of both the metoprolol and atropine-sensitive auricle responses to field stimulation (2 Hz or 4 Hz for 2 s) without affecting the response to exogenous noradrenaline (NA) or acetylcholine (ACh).

NPY did not induce any contraction of the vas deferens, but inhibited both the rapid twitch response and the sustained tonic contraction induced by field stimulation. The NPY-induced inhibition of the tonic contraction was more long-lasting than than of the twitch response. The tonic contraction was blocked by phentolamine and the twitch response by α-, β-methylene ATP tachyphylaxis. NPY did not inhibit the contractile effects of NA, ATP or α-, β-methylene ATP. NPY also induced a reversible reduction of the non-cholinergic, not-adrenergic contractile response to field stimulation of the urinary bladder. In the portal vein, NPY (up to $5x10^{-7}$M) did not inhibit the spontaneous motility or the phentolamine-sensitive contractile responses to field stimulation and NA. The atropine-sensitive contraction of the trachea or the non-adrenergic, non-cholinergic relaxation induced by field stimulation was not significantly influenced by NPY in doses up to $5x10^{-7}$M. In conclusion, the present data show that in the guinea-pig, NPY exerts positive chronotropic and inotropic effects on the right atrium of the heart. Furthermore, NPY may have presynaptic effects on adrenergic, cholinergic and non-adrenergic—non-cholinergic neurotransmission.

Lundberg, J. M., Hua, X.-Y., and Franco-Cereceda, A.

Acta Physiologica Scandinavica 121:325-332, 1984.

Other support: Swedish Medical Research Council, the Swedish Tobacco Company, Astra Foundation, and the Karolinska Institute.

From the Department of Pharmacology, Karolinska Institute, Stockholm, Sweden.

CAPSAICIN-INDUCED STIMULATION OF THE GUINEA-PIG ATRIUM: INVOLVEMENT OF A NOVEL SENSORY TRANSMITTER OR A DIRECT ACTION ON MYOCYTES?

1. The mechanism underlying the positive inotropic and chronotropic effects of capsaicin were investigated using the spontaneously beating guinea-pig atrium *in vitro*.

2. Capsaicin induced a long-lasting stimulatory effect (threshold dose 10^{-9}M). Tetrodotoxin, phentolamine, 6-OHDA, mepyramine plus cimetidine, methysergide-, indomethacin-, somatostatin- or morphine pretreatment and local treatment with capsaicin on the vagal nerves did not reduce the capsaicin response, while it was abolished up to 1 month after systemic capsaicin pretreatment.

3. The capsaicin response was subject to a rapid tachyphylaxis. During capsaicin tachyphylaxis, the positive inotropic and chronotropic effects of noradrenaline, serotonin and histamine were unchanged.

4. Various neuropeptides were investigated with regard to cardiac activity. Physalaemin, eledoisin and somatostatin had negative inotropic and chronotropic effects. Substance P, bombesin, kassinin, CCK-8 or PHI (up to 10^{-6} M of each) did not cause any detectable response on the guinea-pig auricle, while the substance P antagonist [D-Arg, D-Pro, D-Trp, Leu]SP induced a long-lasting stimulation of heart activity. VIP also stimulated the heart.

5. Various adenyl compounds were also tested. Adenosine, AMP, ADP, ATP and β-, γ-methylene ATP had negative chronotopic and inotropic effects, while α-, β-methylene ATP induced a stimulatory response. During α-, β-methylene ATP tachyphylaxis, the auricles still responded to capsaicin. The inhibitory effects of adenosine and ATP analogues were antagonized by theophylline and 8-p-sulfophenyl theophylline. Capsaicin induced a small release of labeled nucleotides from ^{3}(H)-adenine-prelabeled atria from control, but not from capsaicin-pretreated animals.

6. GTP, aspartate and kainic acid (up to 10^{-4}M) had no effect on the guinea-pig atrium, while glutamate had a negative inotropic action.

7. In conclusion, the present findings show a specific stimulatory action of capsaicin on heart function. This effect does not seem to be mediated via any classical transmitter, including substance P and ATP. The capsaicin response was abolished by capsaicin pretreatment, which is known to cause degeneration of chemosensitive nerves in the heart. This suggests that capsaicin may release other bioactive substances than substances P from sensory nerves. A direct action of capsaicin on cardiac myocytes cannot be excluded. A desensitization phenomenon would then also occur on possible receptive sites for capsaicin on the myocytes. Capsaicin pretreatment may thus induce very long-lasting, specific, functional changes in heart function.

Lundberg, J. M., Hua, Y., and Fredholm, B. B.

Archives of Pharmacology 325:176-182, 1984.

Other support: Swedish Medical Research Council, the Swedish Tobacco Company, the Hans och Loo Ostermans Foundation, the Astra Foundation, and the Karolinski Institute.

From the Department of Pharmacology, Karolinska Institute, Stockholm, Sweden.

COMPARATIVE IMMUNOHISTOCHEMICAL AND BIOCHEMICAL ANALYSIS OF PANCREATIC POLYPEPTIDE-LIKE PEPTIDES WITH SPECIAL REFERENCE TO PRESENCE OF NEUROPEPTIDE Y IN CENTRAL AND PERIPHERAL NEURONS

Antisera raised against porcine neuropeptide Y (NPY) and peptide YY (PYY) were characterized with regard to immunohistochemical staining, cross-reactivity to several pancreatic polypeptide (PP)-related peptides, and radioimmunoassayable tissue levels in the rat and pig. The NPY antiserum (102B) reacted with nerves in many areas of both the central and peripheral nervous systems, but it did not stain endocrine cells of the pancreas or intestine. No evidence for any cross-reactivity of the NPY antiserum with related peptides of the PP family, such as avian PP, bovine PP, PYY, γ-MSH, FMRF-amide, or avian PP (31-36), was obtained. The NPY antiserum was N-terminally directed, and regional levels of NPY as seen by radioimmunoassay paralleled well the occurrence of NPY-immunoreactive structures seen in the immunohistochemical study. High pressure liquid chromatography analysis revealed that the NPY-immunoreactive

material from cerebral cortex and vas deferens had elution profiles similar to those of standard porcine NPY. The PYY antiserum mainly stained endocrine cells in the pancreas and intestine as well as a small neuron system in the brainstem of the rat. Although this antiserum had a slight cross-reactivity to NPY in radioimmunoassay, the neuronal PYY staining was separate from that of NPY. High levels of PYY were found in the intestine, and levels above the threshold were also seen in the dorsal vagal complex of the rat. The other antisera investigated (raised against avian PP, bovine PP, γ-MSH, and FMRF-amide) caused neuronal staining that was abolished by preabsorption with NPY. This was also seen even if no detectable cross-reactivity with NPY was found in radioimmunoassay. These latter antisera also stained endocrine cells in the pancreas and intestine with complex cross-reactivity relationships, suggesting the presence of intestinal PP-like peptides in addition to PYY and NPY.

Lundberg, J. M., Terenius, L., Hokfelt, T. and Tatemoto, K.

The Journal of Neuroscience 4/9:2376-2386, 1984.

Other support: Karolinska Institute, Astra Foundation, National Institute of Neurological and Communicate Disorders and Stroke, and the U. S. National Institute of Mental Health.

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CORELEASE OF VASOACTIVE INTESTINAL POLYPEPTIDE AND PEPTIDE HISTIDINE ISOLEUCINE IN RELATION TO ATROPINE-RESISTANT VASODILATION IN CAT SUBMANDIBULAR SALIVARY GLAND

Parasympathetic nerve stimulation of the submandibular salivary gland in the cat caused salivary secretion, vasodilation and a corelease of vasoactive intestinal polypeptide (VIP) and peptide histidine isoleucine (PHI) immunoreactivities (IR) into the venous effluent, as indicated by an increase in output. The ratio between the released VIP-IR and PHI-IR was close to 1:1. Gel-permeation chromatography of plasma from the submandibular venous effluent indicated that the released VIP-IR and PHI-IR were very similar to porcine VIP and PHI, respectively. Atropine pretreatment enhanced output of both VIP-IR and PHI-IR during the parasympathetic nerve stimulation to a similar extent (about 5-fold) compared to control stimulations. This increase could be due to an inhibitory presynaptic muscarinic receptor regulation of VIP and PHI release. Since VIP and PHI are present in the same postganglionic parasympathetic nerves in the gland and both peptides have vasodilator activity, the present data suggest that both VIP and PHI may contribute to the atropine-resistant vasodilation seen upon stimulation of the chorda-lingua nerve. The parasympathetic control of salivary gland function may thus involve a multimessenger system with the classical transmitter acetylcholine and the peptides VIP and PHI.

Lundberg, J. M., Fahrenkrug, J., Larsson, O., and Anggard, A.

Neuroscience Letters 52:37-42, 1984.

Other support: P. Carl Petersen's Fund, NOVO Foundation, Swedish Medical Research Council, Swedish Tobacco Company, Astra Foundation, Swedish Society for Medical Sciences, Karolinska Institute, and Swedish Patent Revenue Research Fund.

From the Department of Pharmacology, Karolinska Institute, Department of Oto-Rhino-Laryngology, Karolinska Hospital, Stockholm, Sweden; Department of Clinical Chemistry, Bispebjerg Hospital, Copenhagen, Denmark.

COMPARISON OF CARDIOVASCULAR AND BRONCHOCONSTRICTOR EFFECTS OF SUBSTANCE P, SUBSTANCE K AND OTHER TACHYKININS

1. The effects of substance P (SP), substance K (SK), physalaemin, eledoisin, kassinin, neuromedin K and bombesin on blood pressure, heart rate, respiratory insufflation pressure and plasma extravasation were studied in the guinea-pig.

2. All tachykinins except neuromedin K caused a fall in blood pressure with rather similar potency. The hypotensive response after physalaemin was comparatively more long-lasting.

3. SK and eledoisin (2.5 nmol × kg^{-1} i.v.) caused an initial bradycardia which then changed into tachycardia. The other tachykinins induced a slowly developing tachycardia. Neuromedin K (up to 40 nmol × kg^{-1}) did not influence heart rate.

4. SK, kassinin and eledoisin were more potent than SP and physalaemin in increasing respiratory insufflation pressure. The effect of SK had a particularly long duration. Neuromedin K only induced a weak increase in insufflation pressure at a very high dose.

5. All tachykinins except neuromedin K induced an increase in vascular permeability to plasma proteins in many visceral organs, as indicated by Evans blue extravasation. The trachea and ureter were the most sensitive organs with regard to this effect. Physalaemin and eledoisin were generally more potent in increasing vascular permeability in various organs than SP and SK. The maximal permeability-increasing effect of SK was smaller than that of SP, although the potency was similar.

6. Bombesin increased insufflation pressure with no clearcut effect on vascular permeability.

7. It is concluded that in the same species, *i.e.*, guinea-pig, several tachykinins have rather similar hypotensive action, while the vascular permeability increase to plasma proteins is especially pronounced after physalaemin and eledoison. SK, kassinin and eledoisin have prominent bronchoconstrictor effects. Neuromedin K, however, displays poor activity in the present models. The existence of novel tachykinins such as SK in addition to SP in mammalian tissues suggests that effects seen upon antidromic stimulation of sensory nerves may be caused by several structurally related peptides.

Hua, X.-Y., *Lundberg, J. M.*, Theodorsson-Norheim, E., and Brodin, E.

Archives of Pharmacology 328:196-201, 1984.

Other support: Swedish Medical Research Council, the Swedish Tobacco Company, the Astra Foundation; and the Karolinska Institute.

From the Department of Pharmacology, Karolinska Institute, and the Department of Clinical Chemistry, Karolinska Hospital, Stockholm, Sweden.

CO-EXISTENCE OF PEPTIDE HI (PHI) AND VIP IN NERVES REGULATING BLOOD FLOW AND BRONCHIAL SMOOTH MUSCLE TONE IN VARIOUS MAMMALS INCLUDING MAN

By immunohistochemistry it was found that PHI- and VIP-like immunoreactivity (IR) occurred in the same autonomic neurons in the upper respiratory tract, tongue and

salivary glands with associated ganglia in rat, guinea pig, cat, pig and man. VIP- and PHI-like immunoreactivity were also found in similar locations in the human heart. The N-terminally directed, but not the C-terminally directed, PHI antiserum or the VIP antiserum stained endocrine cells in the pig duodenum. This suggests the existence of an additional PHI-like peptide. Ligation of nerves acutely caused marked overlapping axonal accumulations of PHI- and VIP-IR central to the lesion. Two weeks after transection of the nerves, both types of immunoreactivities were still observed in accumulations both in the axons as well as in the corresponding cell bodies. The levels of PHI- and VIP-IR in normal tissues from the cat were around 10-50 pmol/g with a molar ratio of about 1 to 2. Systemic administration of PHI and VIP induced hypotension, probably due to peripheral vasodilatation in both guinea pig and cat. Furthermore, both PHI and VIP caused an inhibition of the vagally induced increase in respiratory insufflation pressure in guinea pig. PHI and VIP relaxed the guinea pig trachea *in vitro*, suggesting a direct action on tracheobronchial smooth muscle. VIP was about 5-10 times more potent than PHI with regard to hypotensive effects and 2-3 fold, considering respiratory smooth muscle-relaxant effects in the guinea pig. PHI was about 50-fold less potent in inducing hypotension in the cat than in the guinea pig. Although species differences seem to exist as regards biological potency, PHI should also be considered when examining the role of VIP as an autonomic neurotransmitter.

Lundberg, J. M. et al.

Peptides 5:593-606, 1984.

Other support: Swedish Medical Research Council, Swedish Tobacco Company, NOVO Foundation, Astra Foundation, and the Karolinska Institute.

RELEASE OF SUBSTANCE P- AND SUBSTANCE K-LIKE IMMUNOREACTIVITIES FROM THE ISOLATED PERFUSED GUINEA-PIG LUNG

In this study, the authors offer direct evidence for a release of SP- and SK-like immunoreactivities (LI) by chemical irritation from the isolated perfused guinea-pig lung. Chemical irritation of tissues was achieved by perfusion with buffer containing 1 μM capsaicin. SP-LI was measured with the antibody Rd 2 which does not cross-react to SK. SK-LI was measured with the antibody K12-8307 which does not show cross-reactivity to SP. Infusion of capsaicin induced a several-fold increase in the outflow of SP-LI and SK-LI. Capsaicin, a strong irritant of the respiratory tract, excites and, in high doses, selectively destroys sensory C-fibers. Furthermore, a calcium requiring release of SP from sensory neurons of the guinea-pig ureter by capsaicin has been previously demonstrated. Thus, the present results indicate that SK-LI in the respiratory tract is, like SP, contained in primary afferent C-fibers. Capsaicin-sensitive C-fibers are responsible for both bronchoconstriction and tracheobronchial edema after chemical irritation. SP has been postulated as one mediator of these responses mainly because of the inhibitory effects of SP antagonists which, however, inhibit also actions of other tachykinins.

Saria, A., Theodorsson-Norheim, E., Gamse, R., and *Lundberg, J. M.*
European Journal of Pharmacology 106:207-208, 1985.

Other support: Austrian Scientific Research Fund, Swedish Tobacco Company, Petrus and Augusta Hedlunds Foundation and the Swedish Medical Research Council.

From the Department of Experimental and Clinical Pharmacology, University of Graz, Graz, Austria; Department of Clinical Chemistry, Karolinska Hospital and Department of Pharmacology, Karolinska Institute, Stockholm, Sweden.

DIFFERENTIAL EFFECTS OF RESERPINE AND 6-HYDROXYDOPAMINE ON NEUROPEPTIDE Y (NPY) AND NORADRENALINE IN PERIPHERAL NEURONS

1. The effects of 6-hydroxydopamine (6-OHDA) and reserpine pretreatment on peripheral neuropeptide Y (NPY)- and noradrenaline (NA)-containing neurons were studied in guinea-pigs.

2. Ten days after 6-OHDA pretreatment a 60-80% reduction of the NA content was observed in the right atrium of the heart, stellate ganglion and spleen. The content of NPY-like immunoreactivity (LI) was reduced by about 50% in the heart, and was unchanged in the spleen while it increased to 200% of control in the stellate ganglion. Immunohistochemistry showed a pronounced loss of NPY- and tyrosinehydroxylase (TH)-immunoreactive (IR) nerves in the heart but not in the spleen. Increased NPY-IR was seen in axons and cell bodies of the stellate ganglion.

3. Reserpine pretreatment (threshold dose 0.5 mg X kg^{-1}) caused a dose- and time-dependent reduction of the content of NPY-LI in the heart. A maximal depletion of NPY-LI (about 80%) was observed 5 days after reserpine. Reserpine pretreatment also reduced the content of NPY-LI in the spleen, while no significant change was observed in the adrenal gland or vas deferens. The levels of NPY-LI increased in the stellate ganglion to about 180% of control 5 days after reserpine. Immunohistochemical analysis revealed an almost total loss of NPY-IR nerve fibres in the heart as well as around blood vessels in the lung and skeletal muscle. No detectable changes were observed in perivascular NPY-IR nerves in the spleen, vas deferens or kidney. TH-IR nerves remained unchanged after reserpine, indicating that the observed loss of NPY-IR nerves was due to a depletion of NPY and not a degeneration.

4. No change in the levels of substance P-LI was observed in the right atrium 5 days after reserpine.

5. NA was, in contrast to NPY, markedly depleted in all tissues investigated after reserpine treatment. The depletion of NA was more extensive, and occurred more rapidly and at much lower doses as compared to the effects on NPY-LI.

6. Ligations of the sciatic nerve revealed that NPY-LI was transported axonally with a rapid rate (3 mm/h). Reserpine pretreatment significantly increased the amount of accumulated NPY-IR above the ligation, suggesting an increase in axonal transport.

7. High performance liquid chromatography revealed that the NPY-LI consisted of two major peaks in the stellate ganglia, while only one peak closely corresponding to porcine NPY was seen in the right atrium.

8. In conclusion, 6-OHDA pretreatment depletes NPY-LI in certain terminal regions and increases NPY-LI in ganglia. Reserpine induces a tissue- and dose-dependent depletion of NPY-LI in certain terminal areas, while corresponding cell body content and axonal transport of the peptide seem to increase.

Lundberg, J. M. et al.

Archives of Pharmacology 328:331-340, 1985.

Other support: Swedish Medical Research Council, Swedish Tobacco Company, Astra Foundation, Swedish Society for Medical Sciences, the Karolinska Institute, and Austrian Scientific Research Funds.

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CAPSAICIN PRETREATMENT INHIBITS THE FLARE COMPONENT OF THE CUTANEOUS ALLERGIC REACTION IN MAN

In this study the authors have investigated whether capsaicin pretreatment could impair the cutaneous triple response reaction to antigen challenge in man. The skin of 6 volunteers (3 males, 3 females age 29-40) with an established allergy to rats was pretreated locally with capsaicin. Capsaicin pretreatment produced a burning sensation and flare reaction but no wheal. Injection of rat allergen into control skin area resulted in instant itching followed by a progressively developing flare and wheal reaction. Capsaicin pretreatment of the skin thus almost totally abolished the acute flare component and reduced the itching sensation of the cutaneous allergy reaction in man. This suggests that capsaicin-sensitive sensory nerve endings are activated by mediators released upon mast cell degranulation by allergen exposure. Nerve activation then probably results in the release of vasoactive peptides such as substance P which mediate the flare reaction. The wheal response, however, was unchanged after capsaicin pretreatment. This suggests that activation of sensory nerves plays a major role in the flare and itching reaction while the wheal response is apparently to a major extent independent of mediator release from capsaicin-sensitive nerves.

Lundblad, L., *Lundberg, J. M.*, Anggard, A., and Zetterstrom, O.

European Journal of Pharmacology 113:461-462, 1985.

Other support: Swedish Medical Research Council; Swedish Tobacco Company, and Petrus and Augusta Hedlunds Foundation.

From the Departments of Oto-Rhino-Laryngology and Thoracic Medicine, Karolinska Hospital, and Department of Pharmacology, Karolinska Institute, Stockholm, Sweden.

SUBCELLULAR STORAGE AND AXONAL TRANSPORT OF NEUROPEPTIDE Y (NPY) IN RELATION TO CATECHOLAMINES IN THE CAT

The subcellular storage of neuropeptide Y-like immunoreactivity (NPY-LI) in peripheral sympathetic neurons and adrenal gland as well as its axonal transport in the sciatic nerve was studied in relation to catecholamines in the cat. In the subcellular fractions from different parts of sympathetic neurons, *i.e.*, cell bodies (coeliac ganglia), axons (sciatic nerve) and terminals (spleen), the NPY-LI was found together with noradrenaline (NA) in heavy fractions assumed to contain large dense-cored vesicles. In addition, minor lighter fractions in the coeliac ganglion contained NPY-LI. The molar ratio between vesicular NA and NPY was high in the terminal regions (150 to 1) and much lower in axons and cell bodies (10 to 1), thus reflecting the different mechanisms of resupply for classical transmitter and peptide. In the adrenal gland the NPY-LI was mainly located in the catecholamine-storing chromaffin-granule fraction and also to a smaller extent in lighter fractions. Using reversed-phase HPLC, one molecular from of NPY-LI corresponding to porcine NPY was found in the coeliac ganglion, while the adrenal medulla also contained minor peaks with NPY-LI in addition to the main form,

which co-eluted with porcine NPY. NA was stored both in light and heavy fractions in the spleen, while it was mainly found in heavier fractions in the sciatic nerve. In the coeliac ganglion, most of the noradrenaline was present in a non-particulate form. The anterograde transport rate for NPY-LI in the sciatic nerve was estimated to be about 9 mm h^{-1}. A minor retrograde transport of NPY-LI was also detected. In conclusion, the present data suggest that NPY, a peptide with sympathoactive actions, is co-stored with NA in heavy fractions corresponding to large dense-cored vesicles, while light fractions with small dense-cored vesicles probably contain NA but not NPY-LI. The main resupply of NPY to terminals is, in contrast to NA, most likely by axonal transport, which implicates differences in the storage, turnover and release of these co-existing substances in the sympathoadrenal system.

Fried, G., *Lundberg, J. M.*, and Theodorsson-Norheim, E.

Acta Physiologica Scandinavica 125:145-154, 1985.

Other support: Swedish Medical Research Council, Swedish Society for Medical Sciences, Swedish Tobacco Company, Hedlund's Foundation, and the Karolinska Institute.

From the Department of Physiology and Pharmacology, Karolinska Institute, and Department of Clinical Chemistry, Karolinska Hospital, Stockholm, Sweden.

VIP AND PHI IN CAT NEURONS: CO-LOCALIZATION BUT VARIABLE TISSUE CONTENT POSSIBLE DUE TO DIFFERENTIAL PROCESSING

The concentrations of vasoactive intestinal polypeptide (VIP) and the peptide with NH_2-terminal histidine and COOH-terminal isoleucine (PHI) in various peripheral tissues and some areas in the CNS of the cat were compared with their immunohistochemical localization. The VIP levels in the gastrointestinal tract were 3 to 6 times higher than PHI levels. Much (up to 10-fold) higher VIP than PHI levels were also observed in the genitourinary tract as well as in the lung and heart. In the neurohypophysis, however, the VIP/PHI ratio was close to 1. Gel-permeation chromatography revealed that VIP- and PHI-immunoreactivity (IR) in the intestine, pancreas and brain consisted of three larger molecular forms in addition to the "standard" peptides. These larger forms which had overlapping elution positions may represent prepro-VIP/PHI forms. The immunohistochemical analysis revealed that VIP- and PHI-IR were present in the same ganglion cells in the intestine, pancreas, uterus and sympathetic ganglia. Furthermore, the terminal networks for these two peptides were very similar in the periphery. In the median eminence of the hypothalamus and in the posterior lobe of the pituitary, considerably more nerves were PHI- than VIP-IR. This observation was in parallel to a low VIP/PHI ratio. In conclusion, VIP and PHI seem to co-exist in most neuronal systems. Although the ratio of VIP and PHI on the precursor gene is 1:1, differences in posttranslational processing may create a considerably higher content of VIP than PHI in [illegible] terminal areas.

Fahrenkrug, J., Bek, T., *Lundberg, J. M.*, and Hokfelt, T.

Regulatory Peptides 12:21-34, 1985.

Other support: NOVO Foundation, the P. Carl Petersen's Foundation, the Danish Hospital Foundation for Medical Research, the Swedish Council for Medical Research, the Swedish Tobacco Company, Petrus och Augusta Hedlunds Stiftelse, and the Karolinska Institute.

From the Department of Clinical Chemistry, Bispebjerg Hospital, Copenhagen, Denmark, and the Departments of Pharmacology and Histology, the Karolinska Institute, Stockholm, Sweden.

EVIDENCE FOR SPECIFIC NEUROPEPTIDE Y-BINDING IN RAT BRAIN SYNAPTOSOMES

Neuropeptide Y (NYPY), a neuropeptide with N- and C-terminal tyrosine (Y), is one of the major peptides in the brain and peripheral nervous system. NPY co-exists with noradrenaline (NA) in neurons in both the CNS and periphery but is also present in non-catecholaminergic systems. Sympathetic activation induces co-release of NPY and NA, subsequently these two agents cooperate in functional responses such as vasoconstriction. NPY has therefore been proposed to be a co-transmitter together with NA. In the present study, further evidence is provided for NPY being a neurotransmitter since specific binding sites for ^{125}I-NPY exist in brain membranes of the rat. As demonstrated in this study, the affinity of ^{125}I-NPY to rat brain synptosomes and the number of binding sites are of a magnitude similar to that for receptors described for other putative peptidergic transmitters. ^{125}I-NPY binding could be displaced by unlabeled NPY and PYY, but not by other structurally related peptides from the pancreatic polypeptide family or neurotensin. Therefore, the investigated binding sites show a high specificity for NPY and PYY. However, NPY is probably the endogenous ligand for these binding sites, since the K_i value for NPY was higher than that for PYY and, moreover, PYY does not seem to be present in most areas of the rat brain. The demonstration of specific, high affinity binding sites for ^{125}I-NPY, which have characteristics of pharmacological receptors, further supports the proposal that NPY is an important factor in neurotransmission.

Saria, A., Theodorsson-Norheim, E., and *Lundberg, J. M.*

European Journal of Pharmacology 107:105-107, 1985.

Other supports: Swedish Medical Research Council, Swedish Tobacco Company and Petrus and Augusta Hedlunds Foundation.

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NEUROPEPTIDE Y (NPY): ENHANCEMENT OF BLOOD PRESSURE INCREASE UPON α-ADRENOCEPTOR ACTIVATION AND DIRECT PRESSOR EFFECTS IN PITHED RATS

The effects of neuropeptide Y (NPY) on blood pressure and heart rate were studied in pithed rats. System infusion of NPY in a dose (230 pmol X kg^{-1} X min^{-1}) which *per se* did not affect blood pressure enhanced the pressor response to phenylep... X kg^{-1} i.v.) and that to electrical stimulation of the sympathetic outflow. In higher doses, NPY caused a pressor effect per sen, which was dose-dependently antagonized by nifedipine but not by adrenocaptor antagonists. In conclusion, NPY enhanced the α-adrenoceptor-mediated response and had Ca^{2+}-dependent vasoconstrictor activity *in vivo*.

Dahlof, C., Dahlof, P., and *Lundberg, J. M.*

European Journal of Pharmacology 109:289-292, 1985.

Other support: Swedish Medical Research Council, the Swedish Tobacco Company, the Karolinska Institute, the Swedish Society for Medical Sciences, and the Astra Foundation.

From the Department of Clinical Pharmacology, Sahlgren's Hospital, University of Goteborg, Goteborg, and the Department of Pharmacology, Karolinska Institute, Stockholm, Sweden.

NEUROPEPTIDE Y AND SYMPATHETIC CONTROL OF HEART CONTRACTILITY AND CORONARY VASCULAR TONE

The effects of neuropeptide Y (NPY) on contractility of the spontaneously beating guinea-pig atrium and transmural nerve stimulation (TNS)-induced efflux of tritium-noradrenaline (^{3}H-NA) were studied *in vitro*. NPY induced a moderate positive chronotropic and inotropic atrial response which was resistant to metroprolol. TNA at 2 Hz for 2 s caused an increase in rate and contractile force. These effects were significantly reduced by NPY. NYP also reduced the TNS-induced (2 Hz for 20 s), fractional [^{3}H]NA release by 40% without affecting the contractile response. The contractile effects of exogenous NA on the guinea-pig atrium were not affected by NPY. NPY caused a long-lasting increase in coronary perfusion pressure and also, in high doses, an inhibition of ventricular contractility in the isolated, perfused guinea-pig heart. The perfusion pressure increase to NPY, which most likely reflects coronary vasoconstriction, was resistant to α- and β-adrenoceptor blockade but sensitive to the calcium antagonist nifedipine. A 50% reduction of the vascular NPY response occurred at 10^{-9} M nifedipine, which did not influence cardiac contractility *per se* or the contractile effects of NA. NPY did not modify the increase in ventricular contractility induced by NA. Noradrenaline did not influence coronary perfusion pressure after β-blockade. Since NPY is present together with NA in cardiac nerves, it may be suggested that NPY is involved in the regulation of NA release as well as the sympathetic control of atrial contractility and coronary blood flow.

Franco-Cereceda, A., *Lundberg, L. M.*, and Dahlof, C.

Acta Physiologica Scandinavica 124:361-369, 1985.

Other support: Swedish Medical Research Council, the Swedish Tobacco Company, the Astra Foundation; the Swedish Society for Medical Sciences; and the Karolinska Institute.

From the Department of Pharmacology, Karolinska Institute, Stockholm, and the Department of Clinical Pharmacology, Sahlgren's Hospital, Gothenburg, Sweden.

MECHANISMS UNDERLYING CHANGES IN THE CONTENTS OF NEUROPEPTIDE Y IN CARDIOVASCULAR NERVES AND ADRENAL GLAND INDUCED BY SYMPATHOLYTIC DRUGS

Neuropeptide Y (NPY) is a recently isolated vasoactive peptide which is present, together with catecholamines, in sympathetic nerves and in the adrenal medulla. In the present study, the authors report that pretreatment with sympatholytic agents influences the tissue levels of NPY-like immunoreactivity (NPY-LI) in the guinea pig. Thus, 24 h after reserpine, noradrenaline (NA) and also NPY-LI were depleted in the heart, spleen and the adrenal gland. The levels of NPY-LI in the vas deferens and stellate ganglia, however, were unaffected by reserpine in spite of marked depletions of NA. The reser-

pine-induced depletion of NPY-LI was probably caused by enhanced nerve-impulse flow and subsequent release from cardiovascular nerves in excess of resupply, since it could be prevented by the ganglionic-blocking agent chlorisondamine. Long-term (6 days) treatment with chlorisondamine reduced the levels of NPY-LI in the stellate ganglion. Short-term treatment (48 h) with guanethidine partially prevented the reserpine-induced depletion of NPY-LI, probably due to inhibition of NPY release. Long-term guanethidine treatment depleted not only NA, but also NPY-LI from the spleen. Pretreatment with the alpha-receptor antagonist phenoxybenzamine did not influence the NA levels but reduced the content of NPY-LI in the spleen via a mechanism that was dependent on intact ganglionic transmission. Since NPY has several cardiovascular actions, changes in NPY mechanisms may contribute to the pharmacological and therapeutical effects of sympatholytic agents.

Lundberg, J. M. et al.

Acta Physiologica Scandinavica 124:603-611, 1985.

Other support: Swedish Medical Research Council, Swedish Tobacco Company, Karolinska Institute, Astra Foundation, and the Austrian Scientific Research Funds.

From the Department of Pharmacology, Karolinska Institute, Stockholm, Sweden; the Department of Experimental and Clinical Pharmacology, University of Graz, Graz, Austria; and the Department of Clinical Chemistry, Karolinska Hospital, Stockholm.

NEUROPEPTIDE Y (NPY) REDUCES FIELD STIMULATION-EVOKED RELEASE OF NORADRENALINE AND ENHANCES FORCE OF CONTRACTION IN THE RAT PORTAL VEIN

1. The effect of neuropeptide Y (NPY) on fractional tritium-noradrenaline (^{3}H-NA) release and contractile activity was studied in the isolated portal vein of SHR and WKY rats. 2. NPY (5×10^{-7}M) enhanced the force of the spontaneous contractile activity by about 40%. 3. The fractional ^{3}H-release elicited by transmural nerve stimulation (TNS), which mainly reflects ^{3}H-NA, was reduced by about 40% after preincubation with 5×10^{-7}M NPY in portal veins from both SHR and WKY rats. The inhibitory effect of NPY on TNS-evoked ^{3}H-release was more slowly reversed by washout than the facilitatory action on spontaneous contractile force. 4. The contractile response to field stimulation was not reduced by NPY, but rather tended to be increased. 5. It is concluded that NPY exerts a dual action in the SHR and WKY portal vein, thus enhancing the smooth muscle contractions and inhibiting sympathetic neurotransmission. The inhibitory effect of NPY on TNS-evoked NA efflux, which is present in both SHR and WKY rats, is most likely due to a presynaptic site of action.

Dahlof, C., Dahlof, P., Tatemoto, K., and *Lundberg, J. M.*

Archives of Pharmacology 328:327-330, 1985.

Other support: Swedish Medical Research Council, the Swedish Tobacco Company, Karolinska Institute, and the Astra Foundation.

From the Department of Clinical Pharmacology, Sahlgren's Hospital; Department of Pharmacology, University of Goteborg, Goteborg, Sweden; and the Departments of Biochemistry and Pharmacology, Karolinska Institute, Stockholm, Sweden.

CALCITONIN GENE-RELATED PEPTIDE AND ITS BINDING SITES IN THE HUMAN CENTRAL NERVOUS SYSTEM AND PITUITARY

Binding sites for synthetic human ^{125}I-labeled calcitonin gene-related peptide (^{125}I-CGRP) have been demonstrated in membranes of the human nervous system. Binding was high in the cerebellar cortex (1.35 ± 0.27 fmol/mg of tissue; mean ± SEM), spinal cord (1.06 ± 0.27 to 1.27 ± 0.23 fmol/mg), and nucleus dentatus (1.02 ± 0.15 fmol/mg), intermediate in the inferior colliculus (0.80 ± 0.14 fmol/mg) and substantia nigra (0.75 ± 0.14 fmol/mg), low in the neocortex, globus pallidus, nucleus caudatus, hippocampus, amygdala, superior colliculus, thalamus, and hypothalamus (0.15-0.32 fmol/mg), and negligible in spinal and sympathetic ganglia and pituitary ($\leq$ 0.04 fmol/mg). Autoradiography showed distinct ^{125}I-GRP binding over the molecular and Purkinje cell layers of the cerebellar cortex and over the substantia gelatinosa posterior of the spinal cord. The highest levels of CGRP-like components were recognized in the dorsal part of the spinal cord and the pituitary gland. In the ventral part of the spinal cord as well as in the pituitary and thyroid glands, CGRP values were higher when measured by radioreceptor assay as compared to RIA, indicating that at least two CGRP-like components are present. The predominant CGRP-like peak on HPLC had the retention time of synthetic human CGRP. Immunohistochemistry revealed the presence of a dense plexus of CGRP immunoreactive nerve fibers in the dorsal horn of the spinal cord.

Lundberg, J. M. et al.

Proceedings of the National Academy of Sciences of the United States of America 82:248-252, 1985.

Other support: Swiss National Science Foundation; the Canton of Zurich, Switzerland; Ciba-Geigy Company; Swedish Medical Research Council.

From the Research Laboratory for Calcium Metabolism, Departments of Orthopedic Surgery and Medicine, the Institute of Pathology, University of Zurich, Switzerland; Departments of Histology and Pharmacology, Karolinska Institute, Stockholm, Sweden; and the Department of Anatomy, University of Oxford, Oxford, England.

LEUKOTRIENES C_4, D_4 AND E_4 CAUSE WIDESPREAD AND EXTENSIVE PLASMA EXTRAVASATION IN THE GUINEA PIG

1. Intravenous injection of leukotriene C_4 (1 nmol/kg) caused substantial plasma exudation in anesthetized guinea pigs, as evidenced by marked hemoconcentration (15% 5 min) and significant extravasation of Evans blue.

2. Fluorometric quantitation of Evans blue content in 38 selected tissues documented that leukotriene C_4 caused significant plasma extravasation throughout the body, except for the brain, stomach, duodenum, colon, and gonads.

3. In particular, the respiratory and the uro-genital tracts, but also the conjunctiva, the esophagus, the bile ducts and the umbilical ligaments, were very sensitive to the edema-promoting effect of leukotriene C_4.

4. Intravenous injection of leukotriene D_4 (1 nmol/kg) or E_4 (5 nmol/kg) evoked plasma extravasation with a distribution and magnitude that was similar to that induced by leukotriene C_4.

5. It is concluded that the three major constituents of slow reacting substance of anaphylaxis (SRS-A), leukotrienes C_4, D_4 and E_4 cause a generalized and extensive plasma exudation that is consistent with the proposal that these leukotrienes are important mediators of inflammation.

Hua, X.-Y., Dahlen, S. E., *Lundberg, J. M.*, Hammarstrom, S., and Hedqvist, P.

Archives of Pharmacology 330:136-141, 1985.

Other support: Swedish Medical Research Council; Swedish Society Against Heart and Chest Diseases; Astra Foundation; Harald and Greta Jeanssons Foundation; Swedish Tobacco Company, Petrus and Agusta Hedlunds Foundation; Karolinska Institute; and National Institute of Environmental Medicine.

From the Departments of Pharmacology, Physiology and Physiological Chemistry, Karolinska Institute; and the National Institute of Environmental Medicine, Stockholm, Sweden.

CAPSAICIN TREATMENT DECREASES TISSUE LEVELS OF NEUROKININ A-LIKE IMMUNOREACTIVITY IN THE GUINEA PIG

The tachykinins are a family of naturally occurring bioactive peptides which share a similar C-terminal sequence of amino acid residues and have similar biological activities. Until recently, substance P (SP) was the only tachykinin known to occur in mammalian species. Substance P-like immunoreactivity (SPLI) has been found in the central nervous system and in sensory neurons innervating many peripheral organs. Now, in addition to SP, mammalian tissues have been found to contain tachykinins related to kassinin and SP; neurokinin A (NKA) and neurokinin B (NKB). The purpose of the present study was to investigate whether pretreatment with capsaicin also decreases the concentration of mammalian tachykinins other than SP in some peripheral organs. The results presented here indicate that capsaicin pretreatment depletes certain tissues of tachykinins other than SP. As has previously been shown for SP, the largest relative depletions were found in the tissues containing a high density of primary sensory afferents (ureters, urinary bladder. These results indicate a close relationship between SP and other mammalian tachykinins such as NKA and NKB, not only in terms of similar tissue distribution, but also in terms of similar reactions to capsaicin, suggesting that NKA and NKB are additional peptides of sensory origin. It is therefore interesting that NKA, but not NKB, has a very potent broncho-constrictor action and causes protein extravasation in the tissues, where acute administration of capsaicin reduced the neurokinin concentrations. Neurokinin A may therefore contribute, together with SP, to the neurogenic inflammatory response.

Theodorsson-Norheim, E., Hua, X., Brodin, E., and *Lundberg, J. M.*

Acta Physiologica Scandinavica 124:129-131, 1985.

Other support: Swedish Medical Research Council, Swedish Tobacco Company, Petrus and Margareta Hedlunds Foundation, Magnus Bergvalls Stiftelse, the Astra Foundation, the Nordisk Insulinfond, and the Karolinska Institute.

From the Department of Clinical Chemistry, Karolinska Hospital, and Department of Pharmacology, Karolinska Institute, Stockholm, Sweden.

NON-CHOLINERGIC VASODILATION IN THE TRACHEOBRONCHIAL TREE OF THE CAT INDUCED BY VAGAL NERVE STIMULATION

The tracheobronchial mucosa is extensively vascularized and it also receives a dense parasympathetic and sensory innervation. Chemical or mechanical irritation of the

mucosa induces protective reflexes, such as cough and bronchospasm. In addition, the irritation leads to a clinically and experimentally well-known hyperemia and mucosal swelling. The increased vascular permeability to macromolecules seems to be dependent on activation of sensory nerves which release mediators such as substance P. In the present study, the authors have tried to establish the existence of a vagal non-cholinergic vasodilation in the lower airways using the radioactive microsphere technique to measure blood flow in atropinized cats. The experiments were performed in seven male cats. The data presented in this paper establish the existence of a non-cholinergic vasodilation upon vagal nerve stimulation in the tracheobronchial tree using the microsphere technique. In the nasal mucosa it has been possible to elicit non-cholinergic vasodilation in two principally different ways: (1) by parasympathetic preganglionic nerve stimulation, where the vasodilation is abolished by hexamethonium; and (2) by vasodilation, which is hexamethonium resistant and is elicited by antidromic stimulation of sensory nerves using high threshold stimulation parameters (Lundberg 1984). The parasympathetic non-cholinergic vasodilation is probably due to the release of peptides such as VIP and PHI from postganglionic neurons (Lundberg *et al.* 1984a), while the second type is due to antidromic impulse propagation and the release of tachykinins such as substance P (Lundberg 1984).

Martling, C-R., Anggard, A., and *Lundberg, J. M.*

Acta Physiologica Scandinavica 125:343-346, 1985.

Other support: Swedish Medical Research Council, Swedish Tobacco Company, Petrus and Augusta Hedlunds Foundation, and the Karolinska Institute.

From the Departments of Anesthesiology and Oto-Rhino-Laryngology, Karolinska Hospital, and Department of Pharmacology, Karolinska Institute, Stockholm, Sweden.

NEUROPEPTIDE Y (NPY) AND SYMPATHETIC CONTROL OF BLOOD FLOW IN ORAL MUCOSA AND DENTAL PULP IN THE CAT

Neuropeptide Y (NPY) immunoreactivity (-IR) was found to be present in perivascular nerves in the cat dental pulp and oral mucosa. Many ganglion cells in the superior cervical ganglion also contained NPY-IR. Ligation of the inferior alveolar or lingual nerves produced an accumulation of NPY-IR in axons proximal to the site of ligation, suggesting an anterograde axonal transport of the peptide. After unilateral sympathectomy the NPY-IR disappeared in the dental pulp and oral mucosa on the ipsilateral side. Reversed phase high performance liquid chromatography showed that the main peak of NPY-like immunoreactivity found in the superior cervical ganglion co-chromatographed with synthetic porcine NPY. Changes in blood flow in dental pulp or oral mucosa were measured indirectly by recording local clearance of ^{125}I during electrical stimulation of the sympathetic nerve or during close intra-arterial infusion of noradrenaline or NPY. All three procedures resulted in a pronouced decrease in local blood flow of a similar magnitude in both tissues. After α-adrenoceptor blockade with phentolamine, the vasoconstrictor effect of noradrenaline was abolished. However, the effect of sympathetic stimulation after phentolamine was only partically reduced (23-54%) and the response to NPY was almost unaffected by the α-receptor blockade. The remaining effect of sympathetic stimulation after phentolamine was abolished by guanethidine. However, the response to NPY was not changed by the latter drug. In conclusion, the vasoconstrictor response in the dental pulp and oral mucosa caused by activation of sympathetic nerves is more resistant to phentolamine than the response

induced by infusion of exogenous noradrenaline. Since NPY is probably co-localized with noradrenaline in the sympathetic perivascular nerves and NPY reduces local blood flow, it is proposed that this peptide is involved in sympathetic vascular control in oral tissues.

Edwall, B., Gazelius, B., Fazekas, A., Theodorsson-Norheim, E., and *Lundberg, J. M.*

Acta Physiologica Hungarica 125:253-264, 1985.

Other support: Swedish Medical Research Council; School of Dentistry, Karolinska Institute; Swedish Tobacco Company; Hedlunds Foundation.

From the Department of Pharmacology, Karolinska Institute, Stockholm, Sweden.

PRE- AND POSTJUNCTIONAL EFFECTS OF NPY ON SYMPATHETIC CONTROL OF RAT FEMORAL ARTERY

Neuropeptide Y (NPY)-like immunoreactivity (LI) has recently been demonstrated in perivascular, noradrenergic (NA), sympathetic neurons. NPY is a potent vasoconstrictor agent *in vivo* while the contractile effects of NPY on isolated blood vessels are variable. For this study, therefore, the authors have analyzed possible pre- and postjunctional effects of NPY in relation to noradrenergic mechanisms in the rat femoral artery. The data presented in this paper show that, except for the vasoconstrictor activity *per se*, NPY enhances the contractile effects of NA on arterial vascular smooth muscle. The NPY effect may be related to changes in the influx of extracellular calcium, since it was absent after nifedipine. The contractile response to transmural nerve stimulation (TNS) was also increased somewhat by NPY. However, in the present study, NPY in the same concentration caused a marked (about 50%) reduction of TNS-evoked ^{3}H-NA efflux. Thus, it is striking that a marked depression of stimulus-evoked ^{3}H-NA secretion can occur simultaneously with an enhanced vascular smooth muscle contraction. This indicates that postjunctional contractile and facilitatory mechanisms of NPY have a more profound effect on sympathetic neurotransmission than a 50% reduction of NA release. Thus, NPY may have an important role in the regulation of sympathetic vascular control.

Lundberg, J. M. et al.

Acta Physiologica Scandinavica 123:511-513, 1985.

Other support: Swedish Medical Research Council, the Swedish Tobacco Company, Petrus and Augusta Hedlunds Foundation, the Astra Foundation and the Karolinska Institute.

From the Departments of Pharmacology and Biochemistry, Karolinska Institute, Stockholm, and Department of Clinical Pharmacology, Sahlgren's Hospital, Gothenburg, Sweden.

AMINO ACID UPTAKE BY HUMAN PLACENTA: ALTERATIONS BY NICOTINE AND TOBACCO SMOKE COMPONENTS AND THEIR IMPLICATIONS ON FETAL GROWTH

The fetus of the smoking mother may partially be subjected to amino acid deficits due to the decreased uptake by trophoblast and the transfer of amino acids from trophoblast to fetal circulation.

Sastry, B. V. Rama.

In: Caciagli, F., Giacobini, E. and Paoletti, R. (eds.): *Developmental Neuroscience: Physiological, Pharmacological and Clinical Aspects.* Elsevier Science Publishers, Amsterdam, pp. 137-140, 1984.

Other support: U. S. United States Public Health Service and National Institutes of Health.

From the Department of Pharmacology, Vanderbilt University, Nashville, TN.

EFFECTS OF CALCIUM CHANNEL BLOCKING AGENTS ON MEMBRANE MICROVISCOSITY AND CALCIUM IN THE LIVER OF THE CARBON TETRACHLORIDE TREATED RAT

Membrane microviscosity was determined from the polarized fluorescence of diphenylhexatriene in plasma membranes and microsomes prepared from the liver of carbon tetrachloride treated rats. It was greatly depressed between 12 and 24 hr after the administration of the carbon tetrachloride. Depression of microviscosity was also seen in the liposomes which were prepared from these membranes. There were decreases in phospholipid content and phospholipid methyltransferase activity, but these changes did not appear to explain the decreased microviscosity. A large accumulation of calcium occurred in the liver cells between 12 and 24 hr after the administration of carbon tetrachloride. Chlorpromazine, verapamil and nifedipine, when administered prior to the carbon tetrachloride, partially reduced the later accumulation of calcium and reduced the degree of histological damage observed. When these agents were administered 12 hr after the administration of carbon tetrachloride, they did not reduce the subsequent accumulation of calcium. When administered prior to and 7 hr after carbon tetrachloride, they had a small but potentially significant effect on the microviscosity change. It is suggested that at low levels of microviscosity, a critical threshold may exist below which entry of calcium into the cell is poorly controlled and that calcium channel blocking agents may be ineffective if administered at a time when membrane microviscosity is very low. Tissue calcium accumulation was associated with visible cell damage.

Landon, E. J., Jaiswal, R. K., Kaukam, R. J. and *Sastry, B. V. Rama*

Biochemical Pharmacology 33(22):3553-3560, 1984.

Other support: U. S. Public Health Service.

From the Department of Pharmacology, Vanderbilt University, School of Medicine, Nashville, TN.

CHOLINERGIC PROPERTIES OF CHOLINE ETHERS

Effects of nine choline ethers, $(CH_3)_3N^+CH_2CH(R)\text{-}0\text{-}R'$, on the muscarinic and nicotinic receptors of longitudinal smooth muscle of guinea pig ileum were studied to understand the role of electronic/steric factors at the ether-oxygen in stimulating the cholinergic receptors. Their ED_{50}s to cause contraction of the ileum in presence of hexamethonium (37×10^{-6}M) were 2 to 307 times higher than that of acetylcholine (ACh; 2.9×10^{-7}M). The relative maximal effects of 5 ethers (1.20 to 1.34) were higher than that of ACh (1.0), while 4 exhibited lower maximal effects (< 0.71). These ethers exhibited

no significant inhibition of choline acetyltransferase and cholinesterase activities from the longitudinal muscle at their ED_{50}s. Hexamethonium significantly increased the ED_{50}s of 5 choline ethers. The ED_{50}s of some of the ethers also were significantly increased by treating the muscle with physostigmine (38.5×10^{-8}M) or physostigmine and hexamethonium. Atropine ($> 1 \times 10^{-6}$M) blocked the contractions induced by these ethers. The steric hinderance caused by the β-methyl and/or O-alkyl groups and the electron density around the ether-oxygen are limiting the muscarinic, as well as nicotinic, potencies of these choline ethers. Choline ethers possessing the beta methyl and O-*n*-propyl, *iso*-propyl and *ter*-butyl groups presumably release ACh at a site causing inhibitory potential through a secondary pathway.

Chaturvedi, A. K. and *Sastry, B. V. Rama.*

Archives Internationales de Pharmacodynamie et de Thérapie 277(1):15-27, 1985.

Other support: U.S. Public Health Service, National Institutes of Health and North Dakota Division of the American Cancer Society.

From the Department of Pharmaceutical Sciences/Toxology, College of Pharmacy, North Dakota State University Fargo, and the Department of Pharmacology, School of Medicine, Vanderbilt University, Nashville, TN.

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AUTOREGULATION OF ACETYLCHOLINE RELEASE: ROLE OF SUBSTANCE P AND METHIONINE ENKEPHALIN

An acetylcholine (ACh)-amplification or positive feedback mechanism and a negative feedback mechanism were proposed for release of ACh from cholinergic nerves. The rate of influx of extracellular calcium ions and two endogenous peptides, substance P (SP) and methionine enkephalin (MEK) were also known to regulate ACh release. Therefore, these studies were undertaken to delineate the various components of the above feedback systems.

Mouse cerebral slices were incubated in a Krebs Ringer bicarbonate buffer containing (methyl-^{3}H) choline (0.1 mM; 0.25 Ci/ml) for 60 min. They were filtered, washed, and transferred to a microbath set up for superfusion with the above buffer. The release of ^{3}H-ACh, SP and MEK into the superfusate was measured as described elsewhere. The following results were observed: (a) The rate of ACh release increased initially for the first 5 min and then declined exponentially. The highest rate of release for SP was observed with 2 min preceding the peak release of ACh. A broad peak for the release of MEK followed that of ACh. (b) SP (0.6 μM) increased ACh release (35%) and Ca^{++} uptake (154%). The long acting enkephalin (D-ala-enkephalinamide, 34 nM) decreased the release of ACh (65%) and well as Ca^{++} uptake (48%). (c) 5-Hydroxymethylfurfuryltrimethylammonium (1.9 nM) decreased ACh release by 50% in a medium containing Ca^{++} (2.6 mM). It blocked ACh release induced by K^+ (20 mM) and disteroylphosphatidic acid (43 μM). It increased both spontaneous (14 times) and evoked (2-3 times) release of SP. It decreased spontaneous (16 times) and evoked (20 times) release of MEK. These results suggest that the positive feedback for ACh release may operate through a muscarinic receptor, SP and activation of Ca^{++} influx, and the negative feedback through a muscarinic receptor, MEK and inhibition of Ca^{++} influx.

Sastry, B. V. Rama. Jaiswal, N., and Tayeb, O. S.

In: *Substance P Metabolism and Biological Actions*, Jordan, C.C. and Oehme, P. (eds.): Taylor and Francis, London, 1985, p. 243.

Other support: U. S Public Health Service.

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NICOTINE

The mediation of the behavioral effects associated with nicotine and smoking has been examined in humans and in several animal species. In general, an improvement in behavior or a reduction in stress has been reported. Since metabolism and dosages have been shown to depend on the species examined and on the rate of administration, it is difficult to speculate on the processes in humans that are influenced by nicotine. Aside from the possible addictive properties of nicotine, its behavioral effects suggest that nicotine may function as a neurotransmitter. The action of nicotine on the nervous system may be mediated through a specific binding site as yet poorly identified. The nicotine binding site in brain probably differs from the classical nicotinic cholinergic receptor site as described in the peripheral nervous system, since binding studies with labeled acetylcholine, α-bungarotoxin, or nicotine suggest that these ligands bind to different sites in brain. Nicotine is of particular interest because of its wide use by man, because it is among the compounds that penetrate the brain very rapidly, and because it affects behavior.

Sershen, H.

In: Lajtha, A. (ed.): *Handbook of Neurochemistry, vol.* 9, New York: Plenum Publishing Corporation, 1985, pp. 263-278.

From the Center for Neurochemistry, Nathan S. Kline Research Institute, Ward's Island, New York.

PROTECTION AGAINST 1-METHYL-4-PHENYL-1,2,3,6-TETRAHYDROPYRIDINE NEUROTOXICITY BY THE ANTIOXIDANT ASCORBIC ACID

Administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP; 2 x 8 mg/kg retro-orbital) to BALB/cBy mice reduced [^{3}H]mazindol binding to striatal membranes by 50%. Reactive oxygen derivatives have been suggested to be involved in MPTP neurotoxicity; therefore, these researchers examined the effects of ascorbic acid (an antioxidant). Ascorbic acid (100 mg/kg) given 20 min prior to MPTP administration appreciably prevented the reduction of [^{3}H]mazindol binding. The involvement of oxidative processes in the mechanism of MPTP neurotoxicity may suggest a relationship to the etiology of Parkinson's disease and the possible benefit of treatment with ascorbic acid.

Sershen, H. et al.

Neuropharmacology 24(12):1257-1259, 1985.

From the Center for Neurochemistry, Nathan S. Kline Research Institute, Ward's Island, New York.

COMPARISON OF [^{3}H]NICOTINE AND [^{3}H]ACETYLCHOLINE BINDING IN MOUSE BRAIN: REGIONAL DISTRIBUTION

In a continuing study of nicotine binding sites, we determined the relative amount of nicotine binding and acetylcholine binding in various brain regions of C57/BL and of DBA mice. Although midbrain showed the highest and cerebellum the lowest binding for both [^{3}H]nicotine and [^{3}H]acetylcholine, the ratio of nicotine to acetylcholine binding showed a three-fold regional variation. Acetylcholine inhibition of [^{3}H]nicotine binding indicated that a portion of nicotine binding was not inhibited by acetylcholine. These results indicate important differences between the binding of (±)-[^{3}H]nicotine and that of [^{3}H]acetylcholine.

Sershen, H., Reith, M. E. A., Hashim, A., and Lajtha, A.

Research Communications in Chemical Pathology and Pharmacology **48**(3)345-352, 1985.

From the Center for Neurochemistry, Nathan S. Kline Institute for Psychiatric Research, Ward's Island, New York.

EFFECT OF N-METHYL-4-PHENYL-1,2,3,6-TETRAHYDROPYRIDINE (MPTP) ON AGE-RELATED CHANGES IN DOPAMINE TURNOVER AND TRANSPORTER FUNCTION IN THE MOUSE STRIATUM

Damage to neuronal cells, either during the normal aging process or related to MPTP neurotoxicity, have implicated oxidative free radical mechanisms. Glutathione (a natural reducing agent) is significantly lower in the substantia nigra compared to other brain regions and virtually absent in the nigra of Parkinsonian patients (Perry *et al.*, 1982). If MPTP neurotoxicity involves free radical mediated processes, it was of interest to examine MPTP neurotoxicity in the aged mouse, in which antioxidant mechanisms may be comprised. The present study shows that in MPTP treated animals, [^{3}H]mazindol binding was decreased by 24% more in aged mice as compared to young mice, and a similar increased sensitivity to the neurotoxic effects of MPTP was observed in a decreased dopamine level (26% lower in aged MPTP treated animals versus young MPTP treated animals). Altogether the data indicate MPTP administration to mice results in a critical loss of dopaminergic cells (40-64%), necessitating both an increase in synthesis and in release (turnover) of dopamine from the surviving neurons. MPTP neurotoxicity is greater in the older mouse possibly associated with an age related increase in the susceptibility of dopaminergic cells to oxidative damage; indicating that aged mice can be useful in studying mechanisms of MPTP neurotoxicity.

Sershen, H., Mason, M. F., Hashim, A., and Lajtha, A.

European Journal of Pharmacology **113**:135-136, 1095.

From the Center for Neurochemistry, Nathan S. Kline Institute, Ward's Island, New York.

ACTH PEPTIDES AS ORGANIZERS OF NEURONAL PATTERNS IN DEVELOPMENT; MATURATION OF THE RAT NEUROMUSCULAR JUNCTION AS SEEN BY SCANNING ELECTRON MICROSCOPY

SEM was used to visualize the normal postnatal development of the neonatal rat neuromuscular junction (nmj). Maturational changes evoked by ACTH/MSH 4-10 (10 μg/kg/day IP) or ACTH/MSH 4-9 (Org 2766) (0.01 μg/kg/day IP) were compared to

controls and to pups treated with nicotine during prenatal and postnatal life, or only during the gestation period. Pregnant females received 0.25 mg/kg 2x daily IP; neonates 0.05 mg/kg/day SC. The Desaki and Uehara and Fahim *et al.* methods revealed the nmj on the extensor digitorum muscle to be covered by a delicate drapery of postjunctional folds that surround the immature endplate region. By the second week of postnatal life, these folds become more complex and cover a larger area. Upon maturation the folds descend and invaginate into the muscle fiber. Peptide treatment with either ACTH/MSH 4-10 or ACTH/MSH 4-9 accelerates maturation of the endplate as demonstrated by the increased convolutions of the folds. Similar effects follow nicotine administration. The observed changes in morphology of the developing nmj subjected to nicotine may be mediated through nicotine-evoked ACTH release.

Frischer, R. E., El-Kawa, N. M. and *Strand, F. L.*

Peptides 6(2),13-19, 1985.

Other support: Biomedical Research Support, Grant Program Division of Research Resources, and National Institutes of Health.

From the Biology Department, New York University, New York.

SPECIFIC BINDING AND METABOLISM OF (-)- AND (+)- [^{3}H]-NICOTINE IN ISOLATED RAT HEPATOCYTES AND HEPATOCYTE MEMBRANES

The specific binding of (-)- and (+)- ^{3}H-nicotine, as well as their relative rates of metabolism, were investigated in isolated rat hepatocytes and hepatocyte membranes. A Scatchard plot of both tritiated enantiomers revealed two components, with the higher affinity site having K_d values of 2×10^{-10} and 3×10^{-9} M and B_{max} values of 5×10^{-15} and 4×10^{-16} moles/mg protein for the (-)- and (+)-enantiomers, respectively. The lower affinity site for both enantiomers had a K_d of 4×10^{-9} M and B_{max} of 5×10^{-14} moles/mg protein. The pH optimum of binding was in the higher pH range, in contrast to brain membranes where the optimum was 6.5 involving the protoneated form of nicotine. A good correlation was observed between the pharmacologic potency of a group of nicotine analogues and their ability to bind to intact and hepatocyte membranes. The rate of conversion of nicotine to cotinine in hepatocytes is related to the degree of binding and accumulation. The results are discussed in terms of the possible relationship of the nicotine binding and translocation to its metabolism.

*Abood, L.G. et al.

Archives internationales de Pharmacodynamie et de Therapie 273(1):62-73, 1985.

Other support: DA00464.

From the Center for Brain Research and Department of Biochemistry, University of Rochester Medical Center, Rochester, NY.

EFFECTS OF CHRONICALLY ADMINISTERED NICOTINE AND SALINE ON MOTOR ACTIVITY IN RATS

This study investigated the differential effects of chronically administered nicotine and saline on motor activity on the rat. Nicotine was administered via a subcutaneously implanted osmotic minipump to effect an 8 hour off, 16 hour on flow. Subjects were 48 male and 48 female albino rats, each about 165 days old. Activity was monitored every hour for 192 consecutive hours. Results indicated that the female animals were more active than the males and that animals receiving nicotine were significantly more active on the first two days of drug administration than control animals; however, by the fourth day there were no significant differences between the activity levels of animals that received nicotine and those of control animals.

Cronan, T., Conrad, J., and *Bryson, R.*

Pharmacology Biochemistry & Behavior 22(5):897-899, 1985.

From the Department of Psychology, San Diego State University, San Diego, CA.

CHARACTERIZATION OF ANTIBODIES TO NICOTINE

The researchers studied the specificity of antibodies produced against four different hapten-carrier conjugates: (D,L)-6-(p-aminobenzamido) nicotine-bovine serum albumin (BSA), (D,L)-6-(ε-aminocaparamido) nicotine-BSA, (D,L)-2-(p-aminobenzamido) nicotine-BSA and (D,L)-6-aminonicotine BSA. All of the produced antibodies showed about equal specificity for both D- and L-isomers of nicotine. The carbon length of the spacing molecule between the hapten and carrier molecule did not given substantial influence to the specificity for the pyrolidine portion of the nicotine molecule. The specificity on pyridine portion was increased as the length of the spacing molecule increased. All of the produced antibodies did not cross-react significantly (less that 0.1%) with cotinine, N-methyl pyridinium compounds of (L)-N'nitrosonornicotine. Studies on nicotine levels in the biological fluids of man and rabbits indicated that the radioimmunoassay technique developed in the present studies could detect as low as 2-5 ng/ml.

Castro, A., Monji, N., Ali, H., Bowman, E. R., and McKennis, H.

Biochemical Archives 1(4)173-183, 1985.

From the Department of Pathology, Hormone Research Laboratory, University of Miami School of Medicine, Miami, FL ,and the Division of Biochemical Pharmacology, Medical College of Virginia, Richmond.

"SEMI-RIGID" AND "FLEXIBLE" LINKAGES IN ANTIBODY PRODUCTION FOR DETERMINATION OF NICOTINE

An increasing interest in precise methods for determination of nicotine levels in body fluids has led to development of numerous procedures, all of which have some disadvantages. However, radioimmunoassays and related procedures have proven to have certain unique advantages with small samples and low nicotine concentrations. In the first published study of a radioimmunoassay for determination of nicotine, it was concluded that 2- and 6-aminonicotine were not suitable functionalized nicotine haptens for various reasons. In the present study of the use of racemic aminonicotine as a functionalized hapten, nicotine antibodies suitable for use in nicotine determinations have been produced from antigens in which both "flexible" and "semi-rigid" chains serve to couple racemic 6-aminonicotine to bovine serum albumin.

Castro, A., McKennis, H., Jr., Monji, N., and Bowman, E. R.

Biochemical Archives 1(4):205-214, 1985.

Other support: The American Tobacco Company.

From the Department of Pathology, Hormone Research Laboratory, University of Miami School of Medicine, Miami, FL, and the Division of Biochemical Pharmacology, Medical College of Virginia, Richmond.

PHOSPHORYLATION OF A CHROMAFFIN GRANULE-BINDING PROTEIN BY PROTEIN KINASE C

Protein kinase C was detected in a group of Ca^{2+}-dependent chromaffin granule membrane-binding proteins (chromobindins) on the basis of Ca^{2+}-, phosphatidylserine-,

1,2-diolein-, and phorbol myristate acetate-stimulated histone kinase activity. When the chromobindins were incubated with [γ-^{32}P]ATP, calcium, and phosphatidylserine, ^{32}P was incorporated predominantly into a protein of mass 37 ± 1 kilodaltons (chromobindin 9, or CB9). Phosphorylation of this protein was also stimulated by diolein and phorbol myristate acetate, indicating that it is a substrate for the protein kinase C activity present in the chromobindins. Maximum phosphate incorporation into CB9 in the presence of 1mM calcium, 75 μg/ml of phosphatidylserine, 2.5 μg/ml of diolein, and 12.5 μg/ml of dithiothreitol was 0.53 mol/mol of CB9 in 5 min. Eight ^{32}P-labeled phosphopeptides were resolved in two-dimensional electrophoretic maps of trypsin digests of CB9. Phosphoamino acid analysis revealed that phosphorylation was exclusively on serine (94%) and threonine (6%) residues. Incubation of the chromobindins with chromaffin granule membranes in the presence of [γ^{32}P]ATP resulted in the incorporation of ^{32}P into eight additional proteins besides CB9 that could be separated from the membranes by centrifugation in the presence of ethylene glycol bis(beta-aminoethyl ether)-N,N,N',N'-tetraacetic acid. We suggest that phosphorylation of CB9 or these additional eight proteins may regulate events underlying exocytosis in the chromaffin cell.

Summers, T. A. and *Creutz, C. E.*

The Journal of Biological Chemistry 260(4):2437-2443, 1985.

Other support: National Institutes of Health, National Science Foundation and University of Virginia Diabetes Research and Training Center.

From the Department of Pharmacology, University of Virginia, Charlottesville.

PHOSPHATIDYLINOSITOL-SPECIFIC PHOSPHOLIPASE C ACTIVITY OF CHROMAFFIN GRANULE-BINDING PROTEINS.

Using [U-^{14}C] phosphatidylinositol as substrate, Ca^{2+}-dependent phospholipase C activity was detected in a group of bovine adrenal medullary proteins that bind to chromaffin granule membranes in the presence of Ca^{2+} ("chromobindins," Creutz, C.E., Dowling, L.G., Sando, J.J., Villar-Palasi, C., Whipple, J.H., and Zaks, W.J. (1983) *J. Biol. Chem.* 258, 14664-14674). The activity was maximal at neutral pH and represented an 80- to 240-fold enrichment of adrenal medullary cytosol phospholipase C activity measured at pH 7.3. The stimulation of activity by Ca^{2+} was complex; no activity was present in the absence of calcium, 25% activation occurred at 1 μM Ca^{2+}, and full activation at 5 mM Ca^{2+}. The enzyme bound to chromaffin granule membranes in the presence of 2 mM Ca^{2+} but was released at 40 μM, suggesting that intrinsic enzyme activity may be regulated by [Ca^{2+}] at 1 μM but additional activation at higher concentrations of calcium is seen *in vitro* as a result of Ca^{2+}-dependent binding of the active enzyme to substrate-containing membranes. This enzyme may generate diacylglycerol and phosphorylated inositol to act as intracellular messengers in the vicinity of the chromaffin granule membrane during the process of exocytosis.

Creutz, C. E., Dowling, L, Kyger, E., and Franson, R. C.

The Journal of Biological Chemistry 250(12):7171-7173, 1985.

Other support: National Institutes of Health and National Science Foundation.

From the Department of Pharmacology, University of Virginia, Charlottesville, and the Department of Biochemistry, Medical College of Virginia, Richmond

DIRECT EFFECTS OF ADRENOCORTICOTROPIC HORMONE ON BOVINE ADRENOMEDULLARY CELLS: ADENOSINE 3',5'-MONOPHOSPHATE-DEPENDENT PHOSPHORYLATION OF TYROSINE HYDROXYLASE

Release of adrenal catecholamine by carbachol has been shown to coincide with an increase in intracellular cAMP levels. Bovine adrenal medullary (BAM) cells were prepared and maintained in culture and used to examine the role of cAMP in stimulus-secretion coupling. The addition of ACTH to these cells caused a 10- to 50-fold increase in cellular cAMP without an effect on catecholamine secretion, suggesting cortical cell contamination. Percoll density separation of both BAM cells and adrenal cortical cells revealed that the greatest cAMP responses to ACTH corresponded to the catecholamine-containing cell fractions and not to those density layers where cortical cells sedimented.

BAM cells isolated on Percoll did not metabolize [^{14}C]cholesterol to steroids as would be expected were the ACTH-stimulated cAMP accumulations due to cortical cell contamination of the cultures. ACTH stimulated protein phosphphorylation in ^{32}P-labeled BAM cells in a manner indistinguishable from that induced by carbachol and forskolin. The major soluble phosphoprotein to be affected by these agents had a relative mol wt of 55-57 kdaltons on sodium dodecyl sulfate-gels and corresponded to tyrosine hydroxylase, which is a specific marker enzyme in the adrenal for chromaffin cells.

We propose that bovine adrenal chromaffin cells express ACTH receptors which are coupled to adenylate cyclase. While no acute effect of ACTH was found on catecholamine secretion, ACTH may play a direct role in the regulation of catecholamine synthesis by stimulating the phosphorylation of tyrosine hydroxylase by cAMP-dependent protein kinase.

Michener, M. L., Peach, M. J., and *Creutz, C. E.*

Endocrinology 117(2):730-737, 1985.

Other support: National Institutes of Health, and the National Science Foundation with additional assistance from the University of Virginia Diabetes Research and Training Center and Vascular Smooth Muscle Program Project.

From the Department of Pharmacology, University of Virginia, Charlottesville.

[illegible] OH SCAVENGER IN AQUEOUS SOLUTIONS AND IN BIOLOGICAL SYSTEMS

By using the technique of pulse radiolysis to generate OH radicals, the authors have determined through competition with SCN^-, I^- and $Fe(CN)_6^{4-}$ the reaction rate constant of mannitol with OH radical at pH = 7 to be $(1.8 \pm 0.4) \times 10^9\ M^{-1}\ s^{-1}$. This value justifies the use of mannitol as an OH scavenger. However, in biological systems there is some uncertainty concerning the relevant concentration of this scavenger. The concentration inside the cell may be very different from that in the solution because of active transport on the one hand or through metabolism on the other. Even if the authors can determine the concentration of mannitol inside the cell, they are not able to determine the concentration of the targets where the damage occurs. For all these

reason... they cannot expect that the effect of OH scavengers in biological systems will be proportional to their rate constants with OH.

Goldstein, S. and *Czapski, G.*

International Journal of Radiation Biology 46(6):725-729, 1984.

Other support: U.S. Department of Energy, and Gesellschaft fur Strahlen Forschung, Neuherberg, West Germany.

From the Department of Physical Chemistry, Hebrew University of Jerusalem, Jerusalem, Israel.

KINETICS OF OXIDATION OF CUPROUS COMPLEXES OF SUBSTITUTED PHENANTHROLINE AND 2,2'-BIPYRIDYL BY MOLECULAR OXYGEN AND BY HYDROGEN PEROXIDE IN AQUEOUS SOLUTION

The kinetics and the reaction mechanism of copper(I) complexes of 5-methyl-1,10-phenanthroline, 5-chloro-1,10-phenanthroline, 5-nitro-1,10-phenanthroline, 2,9-dimethyl-1,10-phenanthroline, and 2,2'-bipyridyl with oxygen and hydrogen peroxide have been investigated in aqueous solutions with use of the pulse radiolysis technique. The oxidation by O_2 is second order in the copper(I) complex, while the oxidation by H_2O_2 is first order in the copper(I) complex. Both reactions are first order in oxidants. The kinetic results of the oxidation of copper(I) complexes by oxygen are interpreted by a mechanism that proceeds via a superoxide intermediate.

Goldstein, S. and *Czapski, G.*

Inorganic Chemistry 24(7):1087-1092, 1985.

Other support: Gesellschaft für Strahlen Forschung, Neuherberg and the U.S. Department of Energy.

From the Department of Physical Chemistry, Hebrew University of Jerusalem, Jerusalem, Israel.

THE CONTRIBUTION OF ENDOGENOUS AND EXOGENOUS EFFECTS TO RADIATION-INDUCED DAMAGE IN THE BACTERIAL SPORE

Radical scavengers such as polyethylene glycol 400 and 4000 and bovine albumin have been used to define the contribution of *exogenous* and *endogenous* effects to the gamma-radiation-induced damage in aqueous bacterial suspensions of *Bacillus pumilus* spores. The results indicate that this damage in the bacterial spore is predominantly *endogenous* both in the presence of 1 atmosphere [illegible] and in anoxia.

Jacobs, G. P., Samuni, A., and *Czapski, G.*

International Journal of Radiation Biology 47(6):621-627, 1985.

Other support: U. S. Department of Energy.

From the Department of Pharmacy, School of Pharmacy, Department of Molecular Biology, School of Medicine, and Department of Physical Chemistry, Hebrew University of Jerusalem, Jerusalem, Israel.

EFFECTS OF ETHANOL AND TEMPERATURE ON GLUCOSE UTILIZATION IN THE IN VIVO AND ISOLATED PERFUSED MOUSE BRAIN

A [^{14}C]2-deoxy-D-glucose technique was used to investigate the effects of ethanol and temperature variation on the rate of glucose utilization in the isolated perfused mouse brain. Glucose utilization rates in six regions of the isolated perfused mouse brain at 37°C were similar to the rates in comparable regions of the *in vivo* mouse brain. The temperature dependence of the glucose utilization rate in the perfused mouse brain suggests that a change in the rate-limiting step of cerebral glucose metabolism occurred between 28°C and 32°C. To investigate the possible involvement of temperature-dependent membrane phase transitions, brain perfusions were performed with a medium containing ethanol, which is known to increase membrane fluidity. Perfusion of the mouse brain with a fluid containing 87 mM ethanol (400 mg/dl) resulted in decreases in the glucose utilization rates at 37 but not at 28°C. At 28°C, ethanol produced an increase in glucose utilization in the medulla-pons and in the cerebellum of perfused brains. *In vivo* rates of cerebral glucose utilization in two lines of mice selectively bred for differences in ethanol-induced sleep time indicated that no differences in basal metabolism or following a 4-g/kg dose of ethanol existed between the two lines. Interpretations of ethanol's differential effects on cerebral glucose utilization rates in the isolated perfused mouse brain at 28°C and 37°C are presented along with a discussion of the apparent discrepancies observed when *in vivo* and perfused brains are treated with equivalent concentrations of ethanol.

Towell, J. F., III, and *Erwin, V. G.*

Alcoholism: Clinical and Experimental Research 6(1):110-116, 1982.

Other support: U. S. Public Health Service.

From the Alcohol Research Center, School of Pharmacy, University of Colorado, Boulder.

EFFECTS OF NICOTINE ON β-ENDORPHIN, α MSH, AND ACTH SECRETION BY ISOLATED PERFUSED MOUSE BRAINS AND PITUITARY GLANDS, *IN VITRO*

The effects of nicotine on secretion of the pituitary peptides β-endorphin, α MSH and ACTH were studied using the isolated perfused mouse brain (IPMB) and isolated superfused pituitaries of C3H mice. Nicotine (6.1 μM) stimulated secretion of β-endorphin immunoreactivity from C3H IPMB approximately twofold. Secretion of α MSH immunoreactivity was stimulated approximately two- and sixfold by 6.1 μM and 12.2 μM nicotine, respectively. However, nicotine (6.1 μM) had no direct effect on the secretion of β-endorphin, α MSH, or ACTH immunoreactivities from the isolated superfused pituitaries. The data suggest nicotine acts in the brain to stimulate pituitary secretion of α MSH and β-endorphin. Electrocorticographic (ECoG) activity of the IPMB was monitored. Nicotine induced characteristic ECoG changes including a reduction of input voltage, a biphasic response of rapid desynchronization followed by prolonged synchronization, and seizure at high doses (12.2 μM).

Marty, M. A., *Erwin, V. G.*, Cornell, K., and Zgombick, J. M.

Pharmacology, Biochemistry & Behavior 22:317-325, 1985.

Other support: U. S. Public Health Service.

From the Center for Alcohol Research, School of Pharmacy, University of Colorado, Boulder.

VANADATE ENHANCEMENT OF THE OXIDATION OF NADH BY $O_2^{\cdot-}$: EFFECTS OF PHOSPHATE AND CHELATING AGENTS.

Vanadate markedly stimulates the oxidation of NADH by $O_2^{\cdot-}$. Both phosphate and Tris are inhibitory, but phosphate diminishes the greater inhibitory effect of Tris and thus gives the appearance of stimulating when added to Tris-buffered reaction mixtures. Chelating agents moderately increased the oxidation of NADH but eliminated the much greater catalytic effect of vanadate. Desferal was the most effective of the chelating agents, and could be used to titrate vanadate spectrophotometrically or in terms of the diminution of its catalytic activity. This permitted the demonstration that metavanadate or orthovanadate could form 1:1 complexes with desferal and that orthovanadate was the catalytically active species.

Darr, D. and *Fridovich, I.*

Archives of Biochemistry and Biophysics 243(1):220-227, 1985.

Other support: United States Army Research Office, National Science Foundation, Glenn Foundation, and National Institutes of Health.

From the Department of Biochemistry, Duke University Medical Center, Durham, NC.

ESSENTIALITY OF THE ACTIVE-SITE ARGININE RESIDUE FOR THE NORMAL CATALYTIC ACTIVITY OF Cu,Zn SUPEROXIDE DISMUTASE.

Chemical modification of bovine and yeast Cu,Zn superoxide dismutases with phenylglyoxal diminishes the catalytic activities by ≥ 98% and treatment of these enzymes with butanedione plus borate leads to ≥ 96% inactivation. The activity loss is accompanied by the modification of less than two arginine residues per subunit with no concomitant loss of Cu or Zn. The phenylglyoxal-modified enzymes require at least a 20-fold greater concentration of cyanide for 50% inhibition than do the corresponding native enzymes. Polyacrylamide-gel electrophoresis and activity staining of the phenylglyoxal-inactivated enzymes demonstrate that the residual activity is largely associated with modified forms that bear lower net positive charge than the native superoxide dismutases.

Borders, C. L., Jr., Saunders, J. E., Blech, D. M., and *Fridovich, I.*

Biochemistry Journal 230:771-776, 85.

Other support: Petroleum Research Fund, U. S. Army Research Office, the Natic.. Science Foundation, and the Glenn Foundation.

From the Department of Chemistry, College of Wooster, Wooster, OH, and the Department of Biochemistry, Duke University Medical Center, Durham, NC.

PSEUDOCATALASE FROM *LACTOBACILLUS PLANTARUM*: EVIDENCE FOR A HOMOPENTAMERIC STRUCTURE CONTAINING TWO ATOMS OF MANGANESE PER SUBUNIT

An improved procedure for the isolation of the pseudocatalase of *Lactobacillus plantarum* has been devised, and the quaternary structure and manganese content of this enzyme have been reexamined. Sedimentation equilibrium of the native enzyme at several salt concentrations gave a molecular weight of 172,000. The subunit weight, obtained by sedimentation equilibrium in 6.4M guanidinium chloride, with or without prior reduction and carboxymethylation, was 34 kilodaltons. The amino acid composition indicated 150 Arg + Lys, and after exhaustive tryptic digestion, 32 peptides were resolved. These data suggest that the pseudocatalase is a homopentamer. Cross-linking with dimethyl suberimidate, followed by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate, yielded five major bands, another indication of pentameric structure. The manganese content was found to be 1.8-2.4 per subunit. $s^0_{20,w}$ was found to be 9.6S and $f/f_0 = 1.2$, suggesting a globular structure of Stokes radius 44 Å.

Beyer, W. F., Jr. and *Fridovich, I.*

Biochemistry **24**(23):6460-6467, 1985.

Other support: National Institutes of Health, U. S. Army Research Office, and the National Science Foundation.

From the Department of Biochemistry, Duke University Medical Center, Durham, NC.

THE SALMONELLA/MAMMALIAN MICROSOME MUTAGENICITY TEST: COMPARISON OF HUMAN AND RAT LIVERS AS ACTIVATING SYSTEMS

The mutagenicity of several test compounds was verified by the Salmonella microsome mutagenicity test (Ames test), using both human liver and rat liver (untreated or pretreated with Aroclor 1254) S9 under identical experimental conditions. Aflatoxin B_1, 3-methylcholanthrene, and cigarette-smoke condensate were less mutagenic in the presence of human-liver S9 than in the presence of rat-liver S9 (particularly after treatment with Aroclor 1254). The opposite was observed with 2-aminoanthracene and to a lesser degree with 2-aminofluorene; correlation studies indicate that the two compounds were activated by the same or by very similar enzymes, probably cytochrome P-450s. These results clearly indicate that human-liver S9, as an activating system, behaves differently than rat-liver S9, therefore, it may constitute a useful, additional tool for the study of mutagenicity and, probably, carcinogenicity in man.

Beaune, P., Lemestre-Gornet, R., Kremers, P., Albert, A., and *Gielen, J.*

Mutation Research **156**:139-146, 1985.

Other support: INSERM (France's National Institute of Health and Medical Research).

From the Laboratoire de Chimie Medicale, Institut de Pathologie, Universite de Liege, Liege, Belgium.

MONOCLONAL ANTIBODIES AGAINST HUMAN LIVER CYTOCHROME P-450

Monoclonal hybridomas which produce antibodies against human liver microsomal cytochrome P-450 were developed. Three similar hybridomas produced antibodies which recognized an epitope specific to a family of human P-450 isozymes (P-450$_6$). This epitope was also present on cytochrome P-450 PCN-E (pregnenolone-16α-carbonitrile induced) from rat liver microsomes, but this isozyme differed from the human P-450$_6$ by its molecular weight. These antibodies enabled the authors to quantify cytochrome P-450$_6$ in human liver microsomes and to demonstrate an important quantitative polymorphism in the human liver monooxygenase system.

Beaune, P., Kremers, P., Letawe-Goujon, R., and *Gielen, J. E.*

Biochemical Pharmacology **34**(19):3547-3552, 1985.

Other support: INSERM (France's National Institute of Health and Medical Research).

From the INSERM, Faculte de Medicine Necker, Paris, France.

EVIDENCE FOR REGULATION OF ACTIN SYNTHESIS IN CYTOCHALASIN D-TREATED HEp-2 CELLS

In HEp-2 cells treated with 0.2 or 2.0 μM cytochalasin D (CD), the relative rate of actin synthesis increased for about 12h and then reached a plateau; this increase was suppressed by actinomycin D (AD). When CD was washed from cells which had been treated for 20 h, the elevated rate of actin synthesis declined to the control value within ca 4 h, as the actin-containing cytoskeletal components rearranged by CD recovered their normal morphology. Subsequently, actin synthesis was depressed below control values for a prolonged period; during recovery from 2 h treatment with CD, this depression was of much shorter duration. Re-addition of CD to cells after a 3h recovery period again induced the cytoskeletal alterations characteristic of CD treatment but did not reverse the prior decline in the rate of actin synthesis. In HEp-2 cells treated with cycloheximide during exposure to CD for 20h, the relative rate of actin synthesis measured after removal of cycloheximide was twofold higher than with CD alone and such cells exhibited a twofold slower decline in the rate of actin synthesis during recovery from CD in the continued presence of cycloheximide. These effects of cycloheximide, which resemble observations on "super-induction," suggest that actin synthesis in CD-treated and recovering HEp-2 cells may be regulated by a repressor protein. The possibility that the proposed repressor protein is actin and that actin may thus be a feedback inhibitor of its own synthesis is discussed.

Tannenbaum, J., Brett, J. G. and *Godman, G. C.*

Experimental Cell Research 160:435-448, 1985.

Other support: Muscular Dystrophy Association and National Institutes of Health.

From the National Science Foundation and the Department of Pathology, College of Physicians & Surgeons of Columbia University, New York.

MACROVACUOLATION INDUCED BY CYTOCHALASIN: ITS RELATION TO THE CYTOSKELETON, MORPHOLOGICAL AND CYTOCHEMICAL OBSERVATIONS

At higher doses of cytochalasin (e.g. 3 μg/ml for 3-20 hr), cells of the rat fibroblastoid line, Hmf, undergo extreme retraction, arborization, and subsequent rounding, and develop big cystic vacuoles. These vacuoles are always closely invested by microfilamentous masses, the CD-induced derivatives of the actin-based cytoskeleton, which aggregate in the endoplasm. Vacuolation is progressive (e.g. 12% cells at 6 hr, > 80% at 18 hr), related to total dose (concentration × time) and to congener (CD > CB). Vacuole membranes have the same dimension (85 Å), surface marker 5'nucleotidase, and junctional specializations as those found at the cell surface, they lack the membrane markers associated with endomembrane systems (e.g. AcPase, TPPase, IDPase) and are not lysosomal. Vacuoles represent internalized plasma membrane, they apparently result from retention in the endoplasm, and fusion, of pinocytotic vesicles originating at the cell surface. Vacuole membrane is always in intimate relation to the actin-based microfilament aggregates that surround the vacuoles, and actin-membrane linker proteins fodrin and vinculin are localized at the vacuole boundaries. Vacuoles and their enveloping actin-filament aggregates are surrounded by arrays of vimentin-based intermediate filaments. A new membranous compartment with characteristics of plasma membrane is thus formed within the cell under the influence of CD. Rounding brought about by other means causes no vacuolization. Macrovacuolation, like the other changes caused by CD, is completely reversible on restoration of cells to normal medium.

Brett, J. G. and *Godman, G. C.*

Tissue & Cell 16(3):311-324, 1984.

Other support: National Institutes of Health.

From the Department of Pathology, College of Physicians & Surgeons of Columbia University, New York.

MONOCLONAL ANTIBODIES DETECT THE CONSERVATION OF MUSCARINIC CHOLINERGIC RECEPTOR STRUCTURE FROM *DROSOPHILA* TO HUMAN BRAIN AND DETECT POSSIBLE STRUCTURAL HOMOLOGY WITH α_1-ADRENERGIC RECEPTORS

Muscarinic cholinergic receptors isolated from *Drosophila* heads, rat and human brain, dog heart, and monkey ciliary muscle were examined for structural similarities/differences by utilizing isoelectric focusing, sodium dodecyl sulfate/polyacrylamide gel electrophoresis, and monoclonal antibody cross-reactivity. Muscarinic receptors were affinity labeled with [^{3}H]propylbenzilylcholine mustard and subjected to isoelectric focusing. Muscarinic receptors from each species focused with an isoelectric point of 5.9. The same proteins all migrated with an apparent molecular mass of 80,000 daltons on sodium dodecyl sulfate gels. Six hybridomas secreting monoclonal antibodies specific for muscarinic receptors were developed by using purified rat brain muscarinic receptors as the antigen. The six different monoclonal antibodies immunoprecipitated muscarinic receptors from all tissues and species tested, including human and *Drosophila* brains, with equal efficacy. These data indicate that muscarinic receptors are highly conserved over a considerable evolutionary period. One of the six muscarinic receptor monoclonal antibodies also immunoprecipitated rat liver α_1-adrenergic receptors. Furthermore, two out of five monoclonal antibodies raised

against those receptors immunoprecipitated muscarinic receptors. These data suggest that some degree of structural homology exists between muscarinic cholinergic receptors and α_1-adrenergic receptors.

Venter, J.C., Eddy, B., *Hall, L.M.*, and Fraser, C.M.

Proceedings of the National Academy of Sciences of the United States of America 81:272-276, 1984.

Other support: American Heart Association.

From the Department of Molecular Immunology, Roswell Park Memorial Institute, New York State Department of Health, Buffalo, and the Department of Genetics, Albert Einstein College of Medicine, The Bronx, NY.

α-BUNGAROTOXIN BLOCKS EXCITATORY SYNAPTIC TRANSMISSION BETWEEN CERCAL SENSORY NEURONES AND GIANT INTERNEURONE 2 OF THE COCKROACH, *PERIPLANETA AMERICANA*

1. Autoradiographic localization of an ^{125}I-α-bungarotoxin binding component revealed that specific binding was distributed mainly in the neuropile and to some extent in the periphery of the terminal abdominal ganglion of the cockroach, *Periplaneta americana*.

2. Action potentials recorded from the axon of GI 2 under current-clamp conditions were not affected by exposure to 1.0×10^{-5}M α-bungarotoxin.

3. Excitatory postsynaptic potentials recorded from GI 2, evoked by stimulation of cercal sensory neurones, were sensitive to block by relatively low (from 10^{-9} to 10^{-7}M) concentrations of α-bungarotoxin. The time-course for blockade was found to depend on toxin concentration and frequency of afferent stimulation.

4. Excitatory postsynaptic potentials recorded from GI 2 were not affected by exposure to 1.0×10^{-6}M quinuclidinyl benzilate.

5. We conclude that at least a portion of the ^{125}I-α-bungarotoxin binding component represents cholinergic receptors which have a postsynaptic function at synapses between cercal sensory neurones and GI 2.

Sattelle, D.B., Harrow, I.D., Hue, B., Pelhate, M., Gepner, J.I., and *Hall, L.M.*

Journal of Experimental Biology 107:473-489, 1983.

Other support: Ciba Foundation Anglo-French Exchange Bursary and the U.S. National Science Foundation.

From the A.R.C. Unit of Insect Neurophysiology and Pharmacology, Department of Zoology, Cambridge University, Cambridge, U.K.; Laboratoire de Physiologie, Faculte de Medecine, Universite d'Angers, France; and the Department of Genetics, Albert Einstein College of Medicine, The Bronx, NY.

CHRONIC INHALATION STUDIES IN MICE. I. FACILITIES AND EQUIPMENT FOR "NOSE-ONLY" EXPOSURE TO CIGARETTE SMOKE

Facilities and equipment are described for large-scale, long-term "nose-only" inhalation exposure of mice to whole cigarette smoke. Experimental procedures and

equipment were designed to provide the mice with exposure conditions where (1) the lung was the major target organ for the smoke, (2) large quantities of fresh, whole cigarette smoke could be generated, (3) large numbers of animals could be exposed at one time, (4) routine, daily exposures could be given over a major portion of the lifetime of the animal, (5) monitoring and documentation of the quantity of smoke presented to the animals was provided during each exposure session, (6) safety systems were provided that assured exposure of the animals to smoke only under pre-set exposure conditions, and (7) cigarette smoke was generated under conditions where factors, such as cigarette type, smoke aerosol concentration and smoke particle size, were controlled.

Henry, C. J. et al. (Microbiological Associates)

Beiträge zur Tabakforschung International 13(1):37-53, 1985.

From the Analytical Chemistry Division, Oak Ridge National Laboratory, Oak Ridge, TN; Division of Toxicology and Molecular Biology, Microbiological Associates, Bethesda, MD; and Process and Instruments Corporation, Brooklyn, NY.

CALMODULIN-DEPENDENT NAD KINASE OF HUMAN NEUTROPHILS

NAD kinase from human neutrophils has been partially purified by sequential application of Red Agarose, ion-exchange, and gel-filtration chromatography. The enzyme has a broad pH optimum, 7.0-9.5, is strictly dependent upon the presence of Mg^{2+}, and in the absence of calcium exhibits K_m values of 0.6 and 0.9 mM for NAD and ATP, respectively. NAD kinase activity is extremely sensitive to free calcium concentration, with half-maximal activity observed at free calcium concentrations of approximately 0.4 μM. In cellular extracts, calcium-dependent activation of NAD kinase increases the maximum velocity of the reaction from 2- to 5-fold while not affecting K_m values for NAD and ATP. The activity of the partially purified NAD kinase is stimulated 3.5-fold by the addition of calmodulin in the presence of calcium. This stimulation is inhibited by the addition of 20 μM trifluoperazine to the incubation. These data are interpreted as implicating calmodulin in NAD kinase regulation. The total concentration of NADP + NADPH in the human neutrophil used increased 2.2-fold in response to activation by phorbol myristic acetate. Finally, neutrophil NAD kinase has a M_r, based upon gel filtration, of 169,000.

Williams, M. B. and *Jones, H. P.*

Biochemistry and Biophysics 237(1):80-87, 1985.

Other support: Kroc Foundation.

From the Department of Biochemistry, College of Medicine, University of South Alabama, Mobile.

A COMPARATIVE STUDY OF NEUTROPHIL PURIFICATION AND FUNCTION

Several different methods are currently used by numerous laboratories for the isolation of human neutrophils. These methods include partial purification by dextran sedimentation followed by water lysis, and more complete purification procedures utilizing discontinuous density gradients coupled with dextran sedimentation and in some cases hypotonic lysis. Some investigators refrain from using certain purification schemes because certain steps or reagents used in a particular method might adversely affect the

functional parameter of the neutrophil they wish to measure. In spite of these concerns there has been no systematic comparison of the functional status of neutrophils prepared by the various methodologies. In this study we have compared 4 commonly used methods of isolation with neutrophil function. The results of this study indicate that while neutrophil yield and purity were determined by isolation procedure, all cells were equivalent with regard to chemotactic performance, ability to degranulate, and ability to produce superoxide and hypochlorous acid.

Grisham, M. W., Engerson, T. D., McCord, J. M., and *Jones, H. P.*

Journal of Immunological Methods 82:315-320, 1985.

Other support: The American Heart Association.

From the Department of Biochemistry, College of Medicine, University of South Alabama, Mobile.

ACTIVATION-ASSOCIATED ALTERATIONS IN NEUTROPHIL PYRIDINE NUCLEOTIDE LEVELS: A POTENTIAL REGULATORY ROLE FOR CALCIUM AND CALMODULIN

The concentration of NADP + NADPH in resting human neutrophils has been measured to be 24.0 ± 2.7 10^{-18}mol/cell. Upon activation with opsonized zymosan A, phorbol myristic acetate or *N*-formylmethionylleucylphenylalanine, neutrophil NADP + NADPH pools increase to 80.5, 84.0 and 54.0 × 10^{-18}mol/cell, respectively. These increases in pyridine nucleotide concentration are blocked by the addition of the calcium antagonist 8-(*N,N*-dimethylamino)-octyl-3,4,5-trimethoxybenzoate hydrochloride, while calcium ionophore A23187, in the presence of calcium, will trigger the increase in the absence of other stimuli. Calmodulin antagonists trifluoperazine and *N*-(6-aminohexyl)-5-chloro-1-naphthalene sulfonamide also inhibit stimulus-induced increases in the NADP + NADPH pool. These studies are interpreted as suggesting a role for calcium and calmodulin and possibly protein kinase C in the regulation of pyridine nucleotide concentration in the activated neutrophil.

Sparkman, T. B., Johns, T., Engerson, T., and *Jones, H. P.*

Biochimica et Biophysica Acta 846:8-13, 1985.

Other support: National Institutes of Health.

From the Department of Biochemistry, College of Medicine, University of South Alabama, Mobile.

EFFECT OF ALLOPURINOL ON NEUTROPHIL SUPEROXIDE PRODUCTION, [illegible] OR DEGRANULATION

Recent studies examining the effect of allopurinol on bacterial killing by leukocytes, have been interpreted by those authors as proof that xanthine oxidase is the major superoxide producing enzyme in activated leukocytes. To test the assertion that xanthine oxidase is involved in the production of superoxide by activated human neutrophils, the xanthine oxidase content of neutrophils was measured and the effect of allopurinol on neutrophil functions, including superoxide production, was studied. Neutrophils were found to contain a level of xanthine oxidase insufficient to account for the flux of superoxide associated with neutrophil activation. Allopurinol did not inhibit

superoxide production induced by opsonized zymosan, phorbol myristic acetate or formyl-methionylleucylphenylalanine. Furthermore, neither chemotaxis nor degranulation was affected by allopurinol. Allopurinol was also found ineffective in blocking superoxide-mediated carrageenan-induced foot edema in the rat. These studies are interpreted as evidence that xanthine oxidase is not a major superoxide-generating system in activated neutrophils as has been suggested by others.

Jones, H. P., et al.

Biochemical Pharmacology 34(20):3673-3676, 1985.

Other support: National Institute of Arthritis, Metabolism, Digestive and Kidney Disease and the American Heart Association.

From the Department of Biochemistry, College of Medicine, University of South Alabama, Mobile.

INCREASED BETA-THROMBOGLOBULIN IN THE SERUM OF SMOKERS OCCURS BECAUSE OF INCREASED PLATELET COUNTS.

The amount of β-thromboglobulin (β-TG), a platelet alpha granule protein, released in the serum of smokers by thrombin formed during whole blood clotting was found to be significantly higher in smokers than in nonsmokers. This increase is most likely due to observed increased platelet numbers in smokers, rather than to increased platelet content of β-TG, since the concentration of β-TG per platelet is nearly identical in the two groups. An inverse relationship between release and platelet number was also found.

Longenecker, G.L., et al.

Research Communications in Substances of Abuse 5(2):147-152, 1984.

From the Department of Pharmacology, College of Medicine, University of South Alabama, Mobile.

MECHANISMS FOR REFLEXIVE HYPERTENSION INDUCED BY LOCAL APPLICATION OF CAPSAICIN AND NICOTINE TO THE NASAL MUCOSA

The cardiovascular effects of locally applied nicotine and capsaicin to the nasal mucosa were studied in anaesthetized guinea-pigs. Local application of capsaicin (0.3-30 μM) or nicotine (0.3-30 mM) induced dose-dependent increases in arterial blood pressure, mainly due to an increase in peripheral vascular resistance. The capsaicin and nicotine responses were abolished after local anaesthesia and markedly reduced (to about 20% of control) by combined pretreatment with phentolamine and propranolol, suggesting reflexogenic sympathetic activation. System capsaicin pretreatment abolished the hypertensive effect of capsaicin (30 μM) and reduced the response to nicotine application to about 25% of control ($p < 0.001$). Local capsaicin pretreatment of the nasal mucosa one week earlier also significantly reduced the capsaicin response ($p < 0.05$), while the nicotine-induced increase in blood pressure was not significantly changed. The present findings suggest the presence of two afferent mechanisms in the nasal mucosa which induce hypertension upon chemical irritation. The capsaicin response is dependent upon capsaicin-sensitive afferents. The nicotine response involves

mainly capsaicin-sensitive neurons and, in addition, a minor component which is resistant to capsaicin pretreatment. Thus, the hypertensive effect of nicotine applied locally to the nasal mucosa seems to be mainly mediated via sensory mechanisms other than the sneezing response which is not dependent on capsaicin sensitive nerves.

Lundblad, L., Hua, X.-Y., and *Lundberg, J. M.*

Acta Physiologica Scandinavica 121:277-282, 1984.

Other support: Swedish Medical Research Council, Swedish Tobacco Company, Astra Foundation, and the Karolinska Institute.

From the Department of Pharmacology, Karolinska Institute, and the Department of Oto-Rhino-Laryngology, Karolinska Hospital, Stockholm, Sweden.

EFFECTS OF NICOTINE ON BLASTOCYST DEVELOPMENT PRIOR TO IMPLANTATION IN THE RAT

The following study was undertaken to determine the effects of nicotine treatment on conceptus development during the initial 5 days of pregnancy. In addition, litters were monitored for long-term post-partum effects of nicotine exposure on growth and sexual maturation. To investigate possible modes of nicotine action, plasma progesterone levels were determined in treated (N) *vs* control (C) rats during the initial 5 days of pregnancy, and the effects of the alkaloid on uterine blood flow and intrauterine oxygen tension were measured in pseudopregnant rats. Litter size and birth weight were determined in C vs N rats and body growth subsequently plotted. The onset of puberty in females was determined by noting the day of vaginal opening. Results show that twice daily sc injection of nicotine during the first 5 days of pregnancy retards conceptus growth as indicated by the reduced number of cells per blastocyst. The same treatment had no effect on litter size, birth weight or body growth. However, the day of vaginal opening was delayed. Nicotine treatment generally suppressed plasma progesterone levels during the initial 5 days of pregnancy. Furthermore, a single injection of the alkaloid induced a rapid and sustained reduction in uterine blood flow and a concomitant decrease in intrauterine oxygen. As indicated by this work, nicotine may modify conceptus development by altering reproductive tract function. Nicotine-induced changes in serum progesterone levels and uterine blood flow probably result primarily from the alkaloid's diverse and complex actions on the nervous system. The present results confirm and extend previous investigations reporting effects of pre-implantation nicotine exposure on conceptus development and progesterone secretion. In addition, the present study demonstrates that even a relatively brief exposure to nicotine, *i.e.*, during the initial days of pregnancy is sufficient to modify the timing of puberty in the rat. The delay in onset of puberty suggests long-term nicotine induced modification of CNS function.

Mitchell, J. A. and Hammer, R. E.

In: Caciagli, F., Giacobini, E., and Paoletti, R. (eds.): *Developmental Neuroscience: Physiological, Pharmacological and Clinical Aspects*, New York: Elsevier Science Publishers B.V., 1984, pp. 151-155.

From the Department of Anatomy, Wayne State University School of Medicine, Detroit.

EFFECTS OF NICOTINE ON OVIDUCAL BLOOD FLOW AND EMBRYO DEVELOPMENT IN THE RAT

Nicotine (5.0 mg/kg) was injected (s.c.) twice daily on Day 1 or Days 1-4 or 1-5 of pregnancy. Cumulative doses of nicotine retarded embryo cell cleavage and substantially reduced embryo cell number (saline *vs.* nicotine: 42.5 ± 1.7 *vs* 22.1 ± 1.9 nuclei/embryo, at 12:00 h on Day 5; $P < 0.05$. However, treatment for even 1 day (Day 1) significantly reduced cell number (saline *vs.* nicotine: 42.5 ± 1.7 *vs.* 30.5 ± 0.9, at 12:00 h day on Day 5; $P < 0.01$). Nicotine injection also resulted in a marked and prolonged reduction in oviduct blood flow (pretreatment *vs* 90 min after nicotine: 0.61 ± 0.06 *vs.* 0.37 ± 0.10 ml $min^{-1} g^{-1}$; $P < 0.005$. The results indicate that, in the rat, even a brief exposure to nicotine, the chief alkaloid of tobacco, reduces oviducal blood flow and the rate of embryo cell proliferation. The embryo is therefore susceptible to the effects of nicotine before implantation.

Mitchell, J. A. and Hammer, R. E.

Journal of Reproduction & Fertility 74:71-76, 1985.

Other support: The Research Society of Sigma Xi.

From the Department of Anatomy, Wayne State University School of Medicine, Detroit.

SELECTIVE ACTIVATION OF PARTICULATE GUANYLATE CYCLASE BY A SPECIFIC CLASS OF PORPHYRINS

Guanylate cyclase was activated 3- to 10-fold by hemin in a dose-dependent manner in membranes prepared from homogenates of rat lung, C_6 rat glioma cells, or B103 rat neuroblastoma cells. Maximum activation was observed with 50 to 100 μM hemin with higher concentrations being inhibitory. Activation was observed when Mg^{2+}-GTP but not when Mn^{2+}-GTP was used as the substrate. Increased enzyme activity reflected selective activation of the particulate form of guanylate cyclase; hemin inhibited the soluble form of gyanylate cyclase 70 to 90% over a wide range of concentrations. Activation was not secondary to proteolysis since a variety of protease inhibitors failed to alter stimulation by hemin. Protophorphyrin IX had little effect on particulate guanylate cyclase activity and sodium borohydride almost completely abolished hemin-dependent activation. These data suggest a requirement for the ferric form of the porphyrin-metal chelate for activation. However, agents which interact with the iron nucleus of porphyrins, such as cyanide, had little effect on the ability of hemin to activate guanylate cyclase. The stimulatory effects of hemin were observed in the presence of detergents such as Lubrol-PX, and highly purified particulate enzyme could be activated to the same extent as enzyme in native membranes. These data suggest that the interaction of porphyrins with particulate guanylate cyclase is complex in nature and different from that with the soluble enzyme.

Waldman, S. A., Sinacore, M. S., Lewicki, J. A., Chang, L. Y. and *Murad, F.*

The Journal of Biological Chemistry 259(7):4038-4042, 1984.

Other support: National Institutes of Health and Veterans Administration.

From the Departments of Medicine and Pharmacology, Stanford University, Stanford, CA and Veterans Administration Medical Center, Palo Alto, CA.

HIGHLY PURIFIED PARTICULATE GUANYLATE CYCLASE FROM RAT LUNG. CHARACTERIZATION AND COMPARISON WITH SOLUBLE GUANYLATE CYCLASE

Guanylate cyclase was purified 1000-fold from washed rat lung particulate fractions to a final specific activity of 500 nmoles cyclic GMP produced/min/mg protein by a combination of detergent extraction and chromatography on concanavalin A-Sepharose, GTP-agarose, and blue agarose. Particulate guanylate cyclase has a molecular weight of 200,000 daltons, a Stokes radius of 48Å and a sedimentation coefficient of 9.4, while the soluble form has a molecular weight of 150,000 daltons, a Stokes radius of 44Å, and a sedimentation coefficient of 7.0. Whereas the particulate enzyme is a glycoprotein with a specific affinity for concanavalin A and wheat germ agglutinin, the soluble form of guanylate cyclase did not bind to these lectins. Purified particulate guanylate cyclase did not cross-react with a number of monoclonal antibodies generated to the soluble enzyme. While both forms of the enzyme could be regulated by the formation of mixed disulfides, the particulate enzyme was relatively insensitive to inhibition by cystine. With GTP as substrate, both forms of the enzyme demonstrated typical kinetics, and with GTP analogues, negative cooperativity was observed with both enzyme forms. These data support the suggestion that the two forms of guanylate cyclase possess similar catalytic sites although their remaining structure is divergent, resulting in differences in subcellular distribution, physical characteristics and antigenicity.

Waldman, S. A., Lewicki, J. A., Chang, L. Y. and *Murad, F.*

Molecular and Cellular Biochemistry 57:155-166, 1983.

Other support: National Institutes of Health and Veterans Administration.

From the Departments of Medicine and Pharmacology, Stanford University, Stanford, CA, and Veterans Administration Medical Center, Palo Alto, CA.

ENDOTHELIUM-DEPENDENT AND NITROVASODILATOR-INDUCED RELAXATION OF VASCULAR SMOOTH MUSCLE: ROLE OF CYCLIC GMP

The mechanisms by which endothelium-dependent relaxants and nitrovasodilators cause relaxation of vascular smooth muscle has been reviewed. A model explaining these observations is summarized in Fig. 1. The endothelium-dependent vasodilators through interaction with their appropriate receptors, are thought to activate phospholipase A_2 and cause the release of an unsaturated fatty acid. The released unsaturated fatty acid or a metabolite is thought to be the "endothelial relaxant factor" that interacts with the smooth muscle component to cause relaxation. While the unsaturated fatty acid may be oxidized in either the endothelial cell or smooth muscle cell, the lability of the endothelial relaxant factor suggests that at least some of this processing occurs before its release from the endothelium. The model in Figure 1 suggests that an oxidized fatty acid or a derived free radical is responsible for activation of smooth muscle guanylate cyclase and increases in cyclic GMP levels. As pointed out above, the use of various inhibitors of fatty acid

release and metabolism has not allowed us or others to predict the structure of the active material. To date the best evidence suggests that the unsaturated fatty acid is a product of either the lipoxygenase or P-450 pathways.

Rapoport, R. M. and *Murad, F.*

Journal of Cyclic Nucleotide and Protein Phosphorylation Research 9(4-5):281-296, 1983.

Other support: National Institutes of Health and Veterans Administration.

From the Departments of Medicine and Pharmacology, Stanford University School of Medicine, Stanford, CA, and Veterans Administration Medical Center, Palo Alto, CA.

ATRIAL NATRIURETIC FACTOR SELECTIVELY ACTIVATES PARTICULATE GUANYLATE CYCLASE AND ELEVATES CYCLIC GMP IN RAT TISSUES

A study was done on the effects on guanylate cyclase and cyclic GMP accumulation of a synthetic peptide containing the amino acid sequence and biological activity of atrial natriuretic factor (ANF). ANF activated particulate guanylate cyclase in a concentration- and time-dependent fashion in crude membranes obtained from homogenates of rat kidney. Activation of particulate guanylate cyclase by ANF was also observed in particulate fractions from homogenates of rat aorta, testes, intestine, lung, and liver, but not from heart or brain. Soluble guanylate cyclase obtained from these tissues was not activated by ANF. Trypsin treatment of ANF prevented the activation of guanylate cyclase, while heat treatment had no effect. Accumulation of cyclic GMP in kidney minces and aorta was stimulated by ANF activation of guanylate cyclase. These data suggest a role for particulate guanylate cyclase in the molecular mechanisms underlying the physiological effects of ANF such as vascular relaxation, natriuresis and diuresis.

Waldman, S. A., Rapoport, R. M. and *Murad, F.*

The Journal of Biological Chemistry 259(23):14332-14334, 1984.

Other support: National Institutes of Health and Veterans Administration.

From the Departments of Medicine and Pharmacology, Stanford University School of Medicine, Stanford, CA, and Veterans Administration Medical Center, Palo Alto, CA.

ATRIAL NATRIUR[illegible]IC FACTOR ELICITS AN ENDOTHELIUM-INDEPENDENT RELAXATION AND ACTIVATES PARTICULATE GUANYLATE CYCLASE IN VASCULAR S[illegible] MUSCLE

A 26 amino acid synthetic peptide fragment of atrial natriuretic factor (ANF) relaxed isolated rabbit aortic segments in which the endothelium was either intact or functionally destroyed. The relaxations were temporally associated with increases in levels of cGMP with no change in the levels of cAMP. The ANF-induced increases in cGMP were also observed in aortic segments pretreated with calcium-free buffer or the cGMP phosphodiesterase inhibitor M&B-22,948. Qualitatively similar results were obtained for sodium nitroprusside. ANF selectively activated particulate guanylate cyclase, having no effect on the soluble form of the enzyme. Thus, the direct (endothelium-independent) vasodilator effect of ANF may be mediated via increased tissue levels of cGMP. ANF appears to increase vascular cGMP levels by activation of particulate guanylate cyclase.

Winquist, R. J., Faison, E. P., Waldman, S. A., Schwartz, K. *Murad, F.* and Rapoport, R. M.

Proceedings of the National Academy of Sciences of the United States of America 81: 7661-7664, 1984.

Other support: National Institutes of Health and Veterans Administration.

From the Department of Cardiovascular Pharmacology, Merck Institute for Therapeutic Research, West Point, PA; Departments of Medicine and Pharmacology, Stanford University, Stanford, CA; and Veterans Administration Medical Center, Palo Alto, CA.

MECHANISMS OF ADENOSINE TRIPHOSPHATE-, THROMBIN-, AND TRYPSIN-INDUCED RELAXATION OF RAT THORACIC AORTA

The mechanisms by which adenosine triphosphate, thrombin, and trypsin cause relaxation of vascular smooth muscle were investigated. Relaxation of the rat thoracic aorta with adenosine triphosphate, thrombin, and/or trypsin was associated with increased levels of cyclic guanosine monophosphate in both time- and concentration-dependent manners. Thrombin and trypsin did not alter cyclic adenosine monophosphate levels whereas adenosine triphosphate increased cyclic adenosine monophosphate levels after significant relaxation occurred. Removal of the endothelium abolished adenosine triphosphate-, thrombin-, and trypsin-induced relaxation and the associated increased levels of cyclic nucleotides. Relaxation due to these agents was also inhibited by exposure to nordihydroguaiaretic acid, a lipoxygenase inhibitor, and eicosatetraynoic acid, a lipoxygenase and cyclooxygenase inhibitor. Indomethacin, a cyclooxygenase inhibitor, potentiated relaxation to these agents, whereas the increased levels of cyclic nucleotides due to adenosine triphosphate were unaltered. Bromophenacyl bromide, a phospholipase A_2 inhibitor, decreased relaxation due to adenosine triphosphate, thrombin, and trypsin and the associated increased levels of cyclic nucleotides. Removal of extracellular calcium, which also presumably inhibits phospholipase A_2, prevented the elevated levels of cyclic nucleotides and the inhibitory effects of adenosine triphosphate and trypsin on contraction. In contrast, sodium nitroprusside-induced relaxation and/or increased levels of cyclic guanosine monophosphate were unaltered by nordihydroguaiaretic acid, eicosatetraynoic acid, bromophenacyl bromide, and removal of extracellular calcium. After incubation of intact tissue with ^{32}P-orthophosphate, the patterns of protein phosphorylation caused by adenosine triphosphate, thrombin, and trypsin were indistinguishable from those of acetylcholine, sodium nitroprusside and 8-bromo cyclic guanosine monophosphate. All these agents dephosphorylated myosin light chain. Thus, the present study supports the hypothesis that relaxation induced by adenosine triphosphate, thrombin and trypsin is mediated through the formation of an endothelial factor which elevates cyclic guanosine monophosphate levels and causes cyclic guanosine monophosphate-dependent protein phosphorylation and dephosphorylation of myosin light chain.

Rapoport, R. M., Draznin, M. B., and *Murad, F.*

Circulation Research 255/4:468-479, 1984.

Other support: National Institutes of Health, Veterans Administration, National Research Service Award.

From the Departments of Medicine and Pharmacology, Stanford University School of Medicine, Stanford, CA, and Veterans Administration Medical Center, Palo Alto, CA.

ATRIOPEPTIN I ELEVATES CYCLIC GMP, ACTIVATES CYCLIC GMP-DEPENDENT PROTEIN KINASE AND CAUSES RELAXATION IN RAT THORACIC AORTA

Synthetic atriopeptin II, an atrial natriuretic factor with potent vasodilatory effects, was studied in isolated strips of rat thoracic aorta to determine its actions on contractility, cyclic nucleotide concentrations and endogenous activity of cyclic nucleotide-dependent protein kinases. Atriopeptin II was found to relax aortic strips precontracted with 0.3 μM norepinephrine whether or not the endothelial layer was present. Relaxation to atriopeptin II was closely correlated in a time- and concentration-dependent mannner with increases in cyclic GMP concentrations and activation of cyclic GMP-dependent protein kinase (cyclic GMP-kinase). The threshold concentration for all three effects was 1 nM. Atriopeptin II (10 nM for 10 min) produced an 80% relaxation, an 8-fold increase in cyclic GMP concentrations and a 2-fold increase in cyclic GMP-kinase activity ratios. Atriopeptin II did not significantly alter cyclic AMP concentrations or cyclic AMP-dependent protein kinase activity. These data suggest that cyclic GMP and cyclic GMP-kinase may mediate vascular relaxation to a new class of vasoactive agents, the atrial natriuretic factors. Similar effects have been observed with the nitrovasodilator, sodium nitroprusside, and the endothelium-dependent vasodilator, acetylcholine. Therefore, a common biochemical mechanism of action that includes cyclic GMP accumulation and activation of cyclic GMP-kinase may be involved in vascular relaxation to nitrovasodilators, endothelium-dependent vasodilators and atrial natriuretic factors.

Fiscus, R. R., Rapoport, R. M., Waldman, S. A., and *Murad, F.*

Biochimica et Biophysica Acta **846**:179-184, 1985.

Other support: National Institutes of Health and Veterans Administration.

From the Departments of Medicine and Pharmacology, Stanford University, Stanford, CA; and Veterans Administration Medical Center, Palo Alto, CA.

A TWO-STEP PROCEDURE FOR OBTAINING HIGHLY PURIFIED PARTICULATE GUANYLATE CYCLASE FROM RAT LUNG

Guanylate cyclase (GTP pyrophosphate lyase (cyclizing), EC 4.6.1.2), the enzyme catalyzing the formation of cyclic GMP from GTP, exists in both soluble and membrane-bound forms. These enzymes have been implicated as key regulatory components in a variety of biological events such as secretion and smooth muscle relaxation. Understanding the role these enzymes play in cellular regulation is predicated upon obtaining purified preparations of guanylate cyclase. Several procedures for purifying the soluble enzyme to apparent homogeneity from a variety of tissues have been reported. The particulate enzyme has been purified to apparent homogeneity from sea urchin sperm but, to date, homogeneous preparations of particulate guanylate cyclase from mammalian tissues have not been obtained. Here we report our efforts in purifying particulate guanylate cyclase about 8,200- to 52,000-fold from homogenates of rat lung. Portions of this work have appeared in abstract form.

Waldman, S. A., Chang, L. Y., and *Murad, F.*

Preparative Biochemistry **15**(3):103-119, 1985.

Other support: National Institutes of Health and Veterans Administration.

From the Departments of Medicine and Pharmacology, Stanford University, Stanford, CA, and Veterans Administration Medical Center, Palo Alto, CA.

EFFECTS OF ATRIOPEPTIN ON PARTICULATE GUANYLATE CYCLASE FROM RAT ADRENAL

Atriopeptin II activated particulate guanylate cyclase 5-10-fold in a concentration- and time-dependent fashion in crude membranes obtained from homogenates of rat adrenal cortex or medulla. Similar effects were observed with other atriopeptin analogs. Soluble guanylate cyclase and adenylate cyclase in these preparations were not activated. Accumulation of cyclic GMP in minces of adrenal cortex or medulla was increased 6-8-fold due to atriopeptin II activation of particulate guanylate cyclase. Several thiol-reactive agents blocked the activation of particulate guanylate cyclase, suggesting that free thiol groups on membrane proteins may be important in atriopeptin receptor-guanylate cyclase coupling.

Waldman, S. A., Rapoport, R. M., Fiscus, R. R., and *Murad, F.*

Biochimica et Biophysica Acta 845:298-303, 1985.

Other support: Veterans Administration.

From the Department of Medicine and Pharmacology, Stanford University School of Medicine, Stanford, CA, and Veterans Administration Medical Center, Palo Alto, CA.

ROLE OF CYCLIC-GMP IN RELAXATIONS OF VASCULAR SMOOTH MUSCLE

Relaxation of rat aorta segments with sodium nitroprusside and endothelium-dependent vasodilators, such as acetylcholine, histamine, A23187, ATP, thrombin, and trypsin, is associated with cyclic-GMP (cGMP) accumulation in a concentration- and time-dependent fashion. With rat aorta segments, these agents also increase cyclic GMP-dependent protein-kinase activity and alter the incorporation of ^{32}P into numerous smooth-muscle proteins. Identical patterns of protein phosphorylation were observed with both classes of relaxants on two-dimensional gel electrophoresis and autoradiography. The effects of nitroprusside were observed with or without the endothelium present. In contrast, the effects of the endothelium-dependent agents on all of these parameters (cGMP, cGMP-dependent protein kinase and protein phosphorylation) required the integrity of the endothelium. Various inhibitors of phospholipase and lipoxygenase prevented the effects of the endothelium dependent agents, suggesting that a metabolite of arachidonic acid is the endothelium-relaxant factor and responsible for guanylate-cyclase activation. A smooth-muscle protein with decreased ^{32}P incorporation after treatment with either class of relaxants has been identified as myosin light chain. A model is presented suggesting that the effects of endothelium-dependent vasodilators and directly acting nitrovasodilators converge at the level of guanylate-cyclase activation and cGMP accumulation, which explains the common biochemical and physiological effects on smooth muscle of these two classes of vasodilators.

Murad, F., Rapoport, R. M. and Fiscus, R.

Journal of Cardiovascular Pharmacology 7 (Suppl. 3):S111-S118, 1985.

Other support: National Institutes of Health and Veterans Administration.

From the Departments of Medicine and Pharmacology, Stanford University School of Medicine, Stanford, CA, and Veterans Administration Medical Center, Palo Alto, CA.

EFFECT OF SODIUM-POTASSIUM PUMP INHIBITORS AND MEMBRANE-DEPOLARIZING AGENTS ON SODIUM NITROPRUSSIDE-INDUCED RELAXATION AND CYCLIC GUANOSINE MONOPHOSPHATE ACCUMULATION IN RAT AORTA

The purpose of this study was to investigate the relationship between sodium nitroprusside-induced relaxation, inhibition of the sodium-potassium pump, and cyclic guanosine monophosphate. Exposure of rat thoracic aorta to ouabain, or potassium- or magnesium-free Krebs-Ringer bicarbonate solution, procedures which presumably inhibit the sodium-potassium pump, or to potassium chloride or tetraethylammonium, membrane-depolarizing agents, inhibited relaxation to nitroprusside. These conditions had little or no effect on the elevated cyclic guanosine monophosphate levels at a concentration of nitroprusside (0.1 μM) that relaxed norepinephrine contracted tissues by 80%. However, at a maximum relaxant concentration of nitroprusside (1.0 μM), these conditions decreased the elevation of cyclic guanosine monophosphate. The inhibition of elevated cyclic guanosine monophosphate levels was independent of the endothelium, extracellular calcium, and the cyclic guanosine monophosphate phosphodiesterase inhibitor, M&B 22,948. The inhibitory effects of ouabain and of potassium- and magnesium-free solution on the increased levels of cyclic guanosin monophosphate caused by 1.0 μM nitroprusside were abolished when tissues were incubated without norepinephrine, or with norepinephrine in the presence of the α-adrenergic blocker, phentolamine. In contrast, a β-adrenergic blocker, propranolol, had no effect on the ouabain-induced inhibition of elevated cyclic guanosine monophosphate levels, with norepinephrine present. These results are consistent with the hypothesis that membrane events regulate cyclic guanosine monophosphate synthesis. At nitroprusside concentrations greater than 0.1 μM, the formation of cyclic guanosine monophosphate appears to be coupled to the status of the smooth muscle cell membrane and the integrity of the sodium-potassium pump. Furthermore, the elevated cyclic guanosine monophosphate levels induced by nitroprusside may be compartmentalized and, depending upon the intracellular concentration, may be associated with several functions. These functions may include activation of the sodium-potassium pump and/or membrane hyperpolarization. However, an effect of cyclic guanosine monophosphate on sodium-potassium pump activity and/or membrane potential remains to be demonstrated.

Murad, F., Rapoport, R. M., and Schwartz, K.

Circulation Research 57(1):164-170, 1984.

Other support: National Institutes of Health and Veterans Administration.

From the Departments of Medicine and Pharmacology, Stanford University School of Medicine and Veterans Administration Medical Center, Palo Alto, CA.

CALMODULIN-LANTHANIDE ION EXCHANGE KINETICS

A flow dialysis apparatus suitable for the study of high affinity metal binding proteins has been utilized to study calmodulin-metal exchange kinetics. Calmodulin labeled with Eu-155 and Gd-153 was dialyzed against buffer containing various competing metal ions. The rate of metal exchange was monitored by a gamma-ray scintillation detector. The kinetics of exchange are first order and the rates fall into two categories: Ca(II) and Cd(II) in one, and the lanthanides Eu(III), Gd(III), and LA(III) in the other.

Buccigross, J., O'Donnell, C., and *Nelson, D.*

Science Press, Beijing, China: pp. 406-409, 1985.

From the Department of Chemistry, Clark University, Worcester, MA.

A COMPARATIVE STUDY OF THE DISPOSITION OF NICOTINE AND ITS METABOLITES IN THREE INBRED STRAINS OF MICE

The disposition of nicotine, cotinine and nicotine N-oxide was investigated in male C57BL, DBA, and C3H mice following an ip injection of nicotine (1.0 mg/ml). The half-lives ($t_{\frac{1}{2}}$) of nicotine in blood were 5.9 to 6.9 min. The rapid elimination of nicotine was accompanied by a rapid accumulation of metabolites; maximal concentrations of cotinine in blood (204 to 364 ng/ml) were achieved in 10 min and nicotine N-oxide (23 ng/ml in C3H mice) in 15 min. The $t_{\frac{1}{2}}$ in blood was 20.1 to 39.8 min for cotinine and 18.4 min for nicotine N-oxide. The $t_{\frac{1}{2}}$ values for nicotine in brain were similar to those in blood, but the values for the liver were slightly larger (6.3 to 9.2 min) and interstrain differences were significant. A large strain-related difference in the $t_{\frac{1}{2}}$ for cotinine was found; the metabolite was eliminated from the blood of DBA mice at only about one-half the rate determined for the other strains. The $t_{\frac{1}{2}}$ for nicotine N-oxide in liver ranged from 12.7 to 27.3 min; the values were significantly different with C57BL > DBA and > C3H mice. Strain-related differences were also observed in response to chronic exposure to cigarette smoke. The $t_{\frac{1}{2}}$ of injected nicotine appeared to be slightly decreased in C57BL and DBA mice but was increased by 60% in livers of C3H mice compared to a control group.

Petersen, D. R., Norris, K. J. and Thompson, J. A.

Drug Metabolism and Disposition 12(6):G001-G007, 1984.

From the School of Pharmacy, University of Colorado, Boulder.

ISOLATION AND ANALYSIS OF N-OXIDE METABOLITES OF TERTIARY AMINES; QUANTITATION OF NICOTINE-1'N-OXIDE FORMATION IN MICE

To investigate the formation and elimination of nicotine-1'-N-oxide (NNO) in mice treated with a single injection of nicotine, sensitive and selective methods were developed to quantitate this polar and heat-labile metabolite. The compound was isolated from tissue homogenates as a dodecyl sulfate ion pair with C_{18} extraction cartridges and analyzed on an amino bonded-phase high-performance liquid chromatographic column with a mobile phase consisting of isopropanol-water. Overall recoveries of NNO were 64-76% from biological media. Several methods of detection were evaluated; radiolabeling was necessary to achieve the sensitivity required for

pharmacokinetic studies in mice. The *cis* and *trans* isomers of NNO were separated on a Partisil PAC column and enzymatic selectivity was evaluated for the formation of these isomers in mice.

Thompson, J. A., Norris, K. J. and *Petersen, D. R.*

Journal of Chromatography **341**:349-359, 1985.

From the School of Pharmacy, University of Colorado, Boulder.

INACTIVATION OF HUMAN α-1-PROTEINASE INHIBITOR BY GAS-PHASE CIGARETTE SMOKE

As reported in this study, direct exposure of human α1-proteinase inhibitor (α1PI) to the gas-phase smoke from one cigarette results in a biphasic inactivation of α1PI. (1) The authors observe a rapid, initial loss of elastase inhibitory capacity, the amount of which is dependent upon the age of the smoke. This rapid, *short-term inactivation* is not seen when the protein is exposed to aqueous extracts of cigarette smoke (as had been done in the past). (2) However, both exposure regimens produce a second, *slow inactivation* that occurs over several days. The authors suggest that the short-term inaction may be due to a peroxynitrate (or a similar reactive species) that is formed from radicals in the gas phase but is unstable in aqueous solution. The authors have not yet identified a species responsible for the long-term inactivation observed following both direct exposure to cigarette smoke and exposure to aqueous extracts. One possibility is that the inactivation comes about as a result of the cooxidation of α1PI with autoxidizable smoke compounds that dissolve in aqueous solutions.

Pryor, W. A., Dooley, M. M., and Church, D. F.

Biochemical and Biophysical Research Communications 122(2):676-681, 1984.

Other support: National Institutes of Health and the National Foundation for Cancer Research.

From the Departments of Chemistry and Biochemistry, Louisiana State University, Baton Rouge.

NITROSATION OF ORGANIC HYDROPEROXIDES BY NITROGEN DIOXIDE/DINITROGEN TETRAOXIDE

Cumyl and *tert*-butyl hydroperoxides react rapidly with NO_2 / N_2O_4 in organic solvents in the presence of a base to form the organic nitrate ($RONO_2$) as the major product, together with smaller amounts of the corresponding nitrite (RONO), alcohol, and carbonyl compound (acetophenone or acetone from cumyl and *tert*-butyl hydroperoxide, respectively). The products from *tert*-butyl hydroperoxide are similar whether a base is present or not but those from cumyl hydroperoxide are more complex. The researchers have formulated the initial reaction as a nitrosation of the hydroperoxide by N_2O_4 to give the peroxynitrite ester. This latter species is unstable and either rearranges to give the nitrate or dissociates to form alkoxyl radicals and nitrogen dioxide that ultimately gives the other observed products. The kinetics of the reaction were studied by stopped flow and are complex, but the investigators conclude they are

consistent with the nitrosation mechanism. The rate constants at 30°C are 2.4×10^4 and 8.1×10^3 $M^{-1}s^{-1}$ for *tert*-butyl and cumyl hydroperoxides, respectively. The authors suggest that this facile reaction of NO_2 / N_2O_4 with hydroperoxides may have important consequences with respect to the pulmonary toxicity of NO_2 in smoggy air.

Pryor, W. A., Castle, L., and Church, D. L.

Journal of the American Chemical Society 107(1):211-217, 1985.

Other support: National Institutes of Health.

From the Departments of Chemistry and Biochemistry, Louisiana State University, Baton Rouge.

MECHANISMS OF CIGARETTE SMOKE TOXICITY: THE INACTIVATION OF HUMAN α-1-PROTEINASE INHIBITOR BY NITRIC OXIDE/ISOPRENE MIXTURES IN AIR

A mixture of nitric oxide (NO) and isoprene in air has been studied as a model for gas-phase cigarette smoke. We have shown that this model system duplicates many of the properties of cigarette smoke including the inactivation of human α-1-proteinase inhibitor (α1PI). In this study, buffered solution of α1PI were exposed to puffs of air containing 300 ppm NO and 400 ppm isoprene. Bubbling of the NO/air/isoprene gas stream directly through buffered protein solutions causes α1PI to undergo a fast loss of inhibitory capacity. This fast inactivation is not observed when α1PI is exposed to aqueous extracts of the NO/air/isoprene mixture. Both direct exposure and exposure to aqueous extracts, however, cause α1PI to undergo a slow loss of activity that continues for several days as the protein is incubated in the buffer solutions. Gas-phase cigarette smoke has already been show to cause this same two-phase inactivation of α1PI.

The inactivation of α1PI by the model system is dependent on the presence of oxygen in the gas stream, suggesting that the oxidation of nitric oxide to nitrogen dioxide in air is involved in the formation of the inactivating species. The nature of these species remains to be determined; however, small alkoxyl or peroxyl radicals (such as are spin-trappable from gas-phase smoke as well as from the NO/air/isoprene system) do *not* appear to inactivate α1PI. One possibility is that the inactivating species are metastable compounds formed by radical processes in the gas phase of both cigarette smoke and our model system. Our data suggest that one possible class of species is peroxynitrates.

Pryor, W. A., Dooley, M. M. and Church, D.F.

Biological Interactions 54:173-183, 1985.

Other support: National Institutes of Health and National Foundation for Cancer Research.

From the Departments of Chemistry and Biochemistry, Louisiana State University, Baton Rouge.

INACTIVATION OF HUMAN ALPHA 1-PROTEINASE INHIBITOR BY CIGARETTE SMOKE: EFFECT OF SMOKE PHASE AND BUFFER.

In order to resolve a discrepancy in the literature, we have examined the *in vitro* inactivation of human α1-proteinase inhibitor by direct exposures either to whole cigarette smoke or to filtered (*i.e.*, gas-phase) smoke. Wyss and coworkers reported that whole smoke does not inactivate the protein, whereas we reported that gas-phase smoke does. We now find that direct exposure to gas-phase cigarette smoke causes a slightly greater inactivation of the protein than does direct exposure to whole cigarette smoke, confirming our earlier suggestion that whole smoke is less oxidizing than is gas-phase smoke. This difference, however, does not explain the dramatic difference between our previous findings and those of Wyss and coworkers. The explanation for the discrepancy lies in the nature of the buffers used. Wyss and coworkers used Tris buffer and the use of Tris quenches the activation process almost completely. Our experiments used phosphate buffer. We suggest that Tris is an unsuitable buffer for use in experiments that probe the effects of cigarette smoke.

Pryor, W. A. and Dooley, M. M.

American Review of Respiratory Disease 131:941-943, 1985.

Other support: National Institutes of Health and National Foundation for Cancer Research.

From the Departments of Chemistry and Biochemistry, Louisiana State University, Baton Rouge.

FREE-RADICAL CHEMISTRY OF CIGARETTE SMOKE AND ITS TOXICOLOGICAL IMPLICATIONS

Cigarette smoke contains two very different populations of free radicals, one in the tar and one in the gas phase. The tar phase contains several relatively stable free radicals; we have identified the principal radical as a quinone/hydroquinone (Q/QH_2) complex held in the tarry matrix. We suggest that this Q/QH_2 polymer is an active redox system that is capable of reducing molecular oxygen to produce superoxide, eventually leading to hydrogen peroxide and hydroxyl radicals. In addition, we have shown that the principal radical in tar reacts with DNA *in vitro*, possibly by covalent binding.

The gas phase of cigarette smoke contains small oxygen- and carbon-centered radicals that are much more reactive than are the tar-phase radicals. These gas-phase radicals do not arise in the flame, but rather are produced in a steady state by the oxidation of NO to NO_2, which then reacts with reactive species in smoke such as isoprene. We suggest that these radicals and the metastable products derived from these radical reactions may be responsible for the inactivation of α_1-proteinase inhibitor by fresh smoke.

Cigarette smoke oxidizes thiols to disulfides; we suggest the active oxidants are NO and NO_2. The effects of smoke on lipid peroxidation are complex, and this is discussed. We also discuss the toxicological implications for the radicals in smoke in terms of a number of radical-mediated disease processes, including emphysema and cancer.

Church, D. F. and *Pryor, W. A.*

Environmental Health Perspectives 64:111-126, 1985.

Other support: National Institutes of Health and the National Foundation for Cancer Research.

From the Departments of Chemistry and Biochemistry, Louisiana State University, Baton Rouge.

UTERINE BLOOD FLOW AND CATACHOLAMINE RESPONSE TO REPETITIVE NICOTINE EXPOSURE IN THE PREGNANT EWE

In order to simulate human smoking, experiments were designed to determine what dose of nicotine in the pregnant sheep would produce those plasma nicotine concentrations observed in human smokers and to measure uterine blood flow and plasma catecholamines in response to repetitive exposure (every 30 minutes) to that nicotine dose. Utilizing seven chronically catheterized pregnant sheep equipped with electromagnetic flow probes around both uterine arteries, we observed that a nicotine dose of 0.2 mg/min for 5 minutes results in mean plasma nicotine concentrations of 23.1 ± 1.1 ng/ml SEM (n = 17) immediately following infusion. This dose of nicotine was then infused every 30 minutes for 4 hours, and aliquots of blood were drawn immediately before and after nicotine infusion for determination of plasma catecholamines. No significant alterations in plasma epinephrine and norepinephrine were observed throughout the experiments (n = 8), and no significant changes in uterine blood flow occurred at any time during the experiment (n = 30). We conclude that there is a species difference between sheep and man with respect to the nicotinic threshold for catecholamine release.

Resnik, R., Conover, W. B., Key, T. C., and Van Vunakis, H.

American Journal of Obstetrics 151(7):885-889, 1985.

Other support: U. S. Public Health Service

From the Department of Reproductive Medicine, University of California at San Diego, School of Medicine, and the Department of Biochemistry, Brandeis University, Waltham, MA.

TREATMENT OF DIALYSIS MEMBRANES FOR SIMULTANEOUS DIALYSIS AND CONCENTRATION.

Dialysis membranes used for simultaneous dialysis-concentration required pretreatment to remove uv-absorbing compounds leached from the membranes and to reduce the absorption of protein to the membranes. This was accomplished with sodium carbonate and ethanol or with "sulfur-removal solutions." Protein determinations were made with a micro-Bradford protein reaction and with uv absorbance at 280 nm. Soluble membrane components contributed to [illegible] uv spectra and altered the ratio of 280/260-nm absorbance. Simultaneous dialysis and concentration in the micro protein dialyzer-concentrator apparatus, combining aspects of thin-layer dialysis and ultrafiltration, resulted in rapid removal of salts from the protein solutions. Prior treatment of membranes reduced uncertainties in retentate recoveries, eliminated uv-absorbing components of membranes, and improved recoveries of protein.

Richmond, V. L., St. Denis, R., and Cohen, E.

Analytical Biochemistry 145:343-350, 1985.

From the Pacific Northwest Research Foundation, Seattle, WA.

STRUCTURAL REQUIREMENTS FOR ALLOSTERIC ACTIVATORS OF RAT LIVER MICROSOMAL 3-HYDROXY-3-METHYLGLUTARYL COENZYME A REDUCTASE

Several compounds containing various structural moieties of NAD(P)(H), were examined as possible effectors of rat liver 3-hydroxy-3-methylglutaryl coenzyme A reductase activity. Microsomal reductase was activated with 4.5 mM GSH, assayed with subsaturating NADPH concentration and increasing amounts of the tested compounds. Under these conditions, the essential and sufficient structure required to allosterically enhance the activity of the reductase is that of 5'-AMP. When the 2' position of the nucleotide is phosphorylated, this allosteric activation is diminished.

Roitelman, J. and *Shechter, I.*

Biochemical and Biophysical Research Communications 125(3):902-907, 1984.

From the Department of Biochemistry, the George S. Wise Faculty of Life Sciences, Tel Aviv University, Israel.

ALLOSTERIC ACTIVATION OF RAT LIVER MICROSOMAL 3-HYDROXY-3-METHYLGLUTARYL COENZYME A REDUCTASE BY NICOTINAMIDE ADENINE DINUCLEOTIDES

NADH and NAD^+ are neither substrates nor inhibitors of 3-hydroxy 3-methylglutaryl coenzyme A (HMG-CoA) reductase in concentrations up to 1 mM. Addition of either NADH or NAD^+enhanced the activity of rat liver microsomal reductase, yet NADH failed to affect the activity of the freeze-thaw solubilized enzyme. The degree of enhancement of enzyme activity by NADH decreased as GSH concentration in the assay increased. Addition of 500 μM NADH to the assay converted the sigmoidal (Hill coefficient = 2.0) NADPH-dependent kinetic curve of the microsomal reductase into Michaelis-Menten kinetics (Hill coefficient = 1.1). Furthermore, the kinetic curves were shifted to the left, resulting in an up to 35% decrease in the concentration of NADH required to obtain half-maximal velocity ($S_{0.5}$) in the presence of 500 μM NADH. Again, this effect of NADH was diminished as GSH concentrations increased. These results demonstrate that NAD(H) is an allosteric activator of HMG-CoA reductase. These results also indicate that HMG-CoA reductase has NAD(H) binding site(s) distinct from the catalytic NADPH site(s).

Roitelman, J. and *Shechter, I.*

The Journal of Biological Chemistry 259(22):14029-14032, 1984.

From the Department of Biochemistry, the George S. Wise Faculty of Life Sciences, Tel Aviv University, Israel.

REDUCED GLUTATHIONE IN CHINESE HAMSTER OVARY CELLS PROTECTS AGAINST INACTIVATION OF 3-HYDROXY-3-METHYLGLUTARYL COENZYME A REDUCTASE BY 2-MERCAPTOETHANOL DISULFIDE

When the disulfide of 2-mercaptoethanol (ESSE) is added to the medium of cultured Chinese hamster ovary (CHO) cells, a time and concentration dependent release of 2-mercaptoethanol to the medium is observed. The reduction of ESSE to 2-mercaptoethanol by cells is a saturable process, the rate being approximately 50 nmoles

of 2-mercaptoethanol per mg cell protein for an hour upon exposure to 250 μM ESSE. Reduction rate of ESSE by cells attached to a substratum is independent of glucose and insulin for periods up to 4 hours. However, in detached cells, swirled in suspension, addition of glucose and insulin is necessary in order to obtain a linear reduction rate of ESSE. The rate limiting enzyme in the sterol biosynthetic pathway, 3-hydroxy-3-methylglutaryl Coenzyme A reductase (E.C. 1.1.1.34), is inhibited by ESSE when isolated from CHO cells but total nonsaponifiable lipids synthesis from [2-^{14}C]-acetate in intact cells is not affected by ESSE at concentrations up to 500 μM. Cytosolic reduced glutathinone can spontaneously exchange disulfide bonds with ESSE and thus prevent it from inhibiting the reductase. Cultured cells respond to ESSE administration by elevating their total and acid-soluble glutathione levels. The use of ESSE as a pertubant of the GSH Status in cells is discussed.

Dotan, I. and *Shechter, I.*

Journal of Cellular Physiology 122:14-20, 1985.

From the Department of Biochemistry, the George S. Wise Faculty for Life Sciences, Tel Aviv University, Israel.

ADENOSINE-INDUCED CORONARY RELEASE OF PROSTACYCLIN AT NORMAL AND LOW pH IN ISOLATED HEART OF RABBIT

1. Rabbit hearts were perfused by the Langendorff method with drug-free perfusion medium or with a medium containing adenosine (10^{-7}M - 10^{-3}M) and the coronary and transmyocardial efflux rates of 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$) were measured. Perfusion was performed both at pH 7.4 and 6.9.

2. In other experiments the hearts were pre-labeled with [^{14}C]-arachidonic acid and the coronary efflux of radioactivity and of labeled lipids and 6-keto-$PGF_{1\alpha}$ was determined.

3. The basal coronary flow was elevated by almost 70% during tissue acidosis, in comparison to control. Adenosine induced a dose-dependent increase in the coronary flow, amounting to about 75% at normal pH and a drug concentration of 10^{-5}M. The adenosine-induced increase in coronary flow was not facilitated by low pH.

4. The base coronary efflux of 6-keto-$PGF_{1\alpha}$ from the hearts was 2.5-3.6 ng/min. Adenosine (10^{-6}-10^{-5}M) significantly facilitated this efflux, up to 6.5 ng/min. The efflux of 6-keto-$PGF_{1\alpha}$ was not changed by perfusion with acidic medium, either in the basal state or during perfusion with adenosine.

5. The basal interstitial efflux of 6-keto-$PGF_{1\alpha}$ was 4.5-5.5 ng/min. This efflux was not affected by perfusion of the heart with adenosine-containing medium. In hearts pre-labeled with [^{14}C]-arachidonic acid, adenosine (10 μM) induced a specific liberation of labeled lipid-extractable substances, including 6-keto-$PGF_{1\alpha}$.

From these data we conclude that adenosine stimulates the liberation of 6-keto-$PGF_{1\alpha}$ from the rabbit heart by increasing precursor availability and subsequent formation of prostacyclin in the coronary vessels. Furthermore, the increase in coronary flow induced by tissue acidosis is not related to an augmented formation of prostacyclin.

Ciabattoni, G. and *Wennmalm, Å.*

British Journal of Pharmacology 85:557-563, 1985.

Other support: Swedish Medical Research Council.

From the Department of Pharmacology, Catholic University, Rome, Italy, and the Department of Clinical Physiology at Karolinska Institute, Huddinge University Hospital, Stockholm, Sweden.

CHARACTERIZATION OF PROTEIN KINASE C ACTIVITY IN INTERFERON GAMMA TREATED MURINE PERITONEAL MACROPHAGES

Macrophage activation for tumoricidal microbicidal functions can be achieved in part by treatment with recombinant interferon gamma (IFNγ) *in vitro*. We have previously demonstrated that IFNγ treatment of murine peritoneal macrophages results in a two- to five-fold increase in the activity of Ca^{++} phospholipid dependent protein kinase C. We now report that this effect was not dependent upon continuing protein synthesis, since treatment with cycloheximide under conditions where normal protein synthesis was inhibited by greater than 95% had no effect upon the development of increased enzyme activity. Examination of Ca^{++} and phospholipid requirements revealed no differences between enzyme isolated from control or IFNγ-treated cells could not be distinguished in terms of the diacyglycerol (DC) or phorbol diester (PMA) concentration required for stimulation of activity. Kinetic analysis of the ATP (as substrate) concentration dependence revealed that both control and treated enzyme preparations (either basal or stimulated) had comparable Km values. Maximum velocity (Vmax) was increased both by IFNγ treatment and also by stimulation with DG or PMA. The major difference which could be discerned between protein kinase C derived from control versus IFNγ-treated macrophages was the magnitude of the response to DG or PMA; IFNγ treatment increased the stimulation index (*i.e.*, ratio of basal to stimulated activity) by a factor of two- to four-fold. These results suggest that IFNγ treatment leads to reversible modulation of existing protein kinase C resulting in increased catalytic activity when exposed to an appropriate stimulant.

Becton, D.L., *Adams. D.O.*, and Hamilton, T.A.

Journal of Cellular Physiology 125:485-491, 1985.

Other support: United States Public Health Service.

From the Departments of Pediatrics, Pathology and Microbiology-Immunology, Duke University, Durham, NC.

IDENTIFICATION OF THE LYMPHOKINE SOLUBLE IMMUNE RESPONSE SUPPRESSOR IN URINE OF NEPHROTIC CHILDREN

Patients with minimal change nephrotic syndrome (MCNS) frequently have suppressed *in vivo* and *in vitro* immune responsiveness of uncertain etiology. Because increased suppressor cell activity has been associated with this disease, urines from MCNS patients were screened for activity of the lymphokine soluble immune response suppressor (SIRS), a product of concanavalin A- or interferon-activated suppressor T cells. Urines from untreated MCNS patients suppressed polyclonal plaque-forming cell responses of cultured splenocytes. This suppressive activity was identified as human SIRS by the following functional and physical criteria: (a) molecular weight estimated by gel filtration; (b) kinetics of suppression; (c) inhibition of suppression by catalase, levamisole, and 2-mercaptoethanol; (d) abrogation of activity by acid or protease treatment; (e) elution pattern on high performance liquid chromatography; and (f) cross-reactivity with monoclonal antimurine SIRS antibodies. Suppressive activity disappeared from urine after initiation of treatment but before remission of symptoms.

Urines were tested from 11 patients with MCNS, all of whom excreted SIRS. In addition, two nephrotic patients with acute glomerulonephritis and three nephrotic patients with membranoproliferative disease excreted SIRS, but other nephrotics and all nonnephrotic patients did not. These results indicate that excretion of SIRS occurs in certain cases of nephrotic syndrome and that the presence of SIRS in the urine is not accounted for solely by the presence of proteinuria or nephrosis. Serum from four nephrotic patients also contained SIRS, whereas neither serum nor urine from six normal subjects had SIRS activity. The systemic presence of SIRS in these four patients and the identification of SIRS in urines from a larger group of patients suggest a possible role for SIRS in the suppressed immune responses often found in nephrotic syndrome.

Schnaper, H.W. and *Aune, T.M.*

Journal of Clinical Investigation 76:341-349, 1985.

Other support: Monsanto Company.

From the Department of Pathology and Laboratory Medicine, The Jewish Hospital of St. Louis and the Departments of Pediatrics and Pathology, Washington University School of Medicine, St. Louis.

INHIBITION OF SOLUBLE IMMUNE RESPONSE SUPPRESSOR ACTIVITY BY GROWTH FACTORS

Soluble immune response suppressor (SIRS), a protein of M_r 14,000, is a lymphokine produced by interferon- or concanavalin A-activated suppressor T cells and is oxidized to its activated form, $SIRS_{ox}$, by hydrogen peroxide produced by macrophages. $SIRS_{ox}$ inhibits antibody secretion by B lymphocytes and cell division by normal or transformed cell lines. Effects of purified growth factors on suppression of antibody secretion were examined to determine whether any would oppose the inhibitory effects of SIRS or $SIRS_{ox}$. Interleukin 1 (IL-1), interleukin 2 (IL-2) and epidermal growth factor (EGF) each inhibited SIRS-mediated suppression of antibody secretion by cultured mouse spleen cells. Inhibition of SIRS activity was most effective when growth factors were added late in the culture period. IL-1, IL-2 and EGF also blocked suppression by $SIRS_{ox}$. However, EGF and IL-1 blocked suppression by $SIRS_{ox}$ only when added 3-6 hr before addition of $SIRS_{ox}$, whereas IL-2 blocked suppression by $SIRS_{ox}$ when added before or up to 3 hr after addition of $SIRS_{ox}$. Further evaluation showed that IL-2, but not IGF or IL-1, reversed inhibition of antibody secretion by $SIRS_{ox}$ in a time- and concentration-dependent manner. With 50 units of IL-2 per 0.5-ml culture, reversal was complete within 1 hr. The ability of growth factors to interfere with inhibition of cell division by $SIRS_{ox}$ was examined with the human B-cell leukemia RPMI-1788. This cell line binds EGF but is not known to have cell surface receptors for IL-1 or IL-2. EGF (0.3-1 ng/ml), when added to RPMI-1788 cultures 4-6 hr before $SIRS_{ox}$, interfered with the ability of $SIRS_{ox}$ to inhibit cell division. Taken together, these data indicate that growth factors interfere with both the immunosuppressive and growth inhibitory properties of $SIRS_{ox}$ in both heterogeneous and homogeneous cell populations.

Aune, T.M.

Proceedings of the National Academy of Sciences of the United States of America 82:6260-6264, 1985.

Other support: Science Foundation and the Monsanto Company.

From the Department of Pathology and Laboratory Medicine, The Jewish Hospital of St. Louis; and the Department of Pathology, Washington University School of Medicine, St. Louis.

PRODUCTION OF THE LYMPHOKINE SOLUBLE IMMUNE RESPONSE SUPPRESSOR (SIRS) DURING CHRONIC EXPERIMENTAL SCHISTOSOMIASIS MANSONI

Chronic schistosomiasis mansoni is a helminthic infection characterized by cell-mediated, anti-egg granulomatous reactions and a variety of associated immunoregulatory phenomena. Soluble immune response suppressor (SIRS) is a lymphokine produced by activated suppressor T lymphocytes in various experimental settings. This report demonstrates the presence of SIRS in the sera of mice with chronic schistosomiasis mansoni (at least 20 wk of infection), but not in the sera of mice with earlier infections. Also, cultures of isolated, intact, hepatic, egg-focused granulomas from chronically infected mice release detectable levels of SIRS. These are the immunomodulated lesions characteristic of this infections. Large, intense, unmodulated granulomas obtained from acutely infected mice did not release SIRS. There is, therefore, a strong association between the presence of SIRS in the serum, the production of SIRS by intact lesions, and the chronic, immunomodulated stage of schistosomiasis mansoni.

Aune, T.M., Freedman, G.L. Jr., and Colley, D.G.

The Journal of Immunology 135(4):2768-2771, 1985.

Other support: National Science Foundation, National Institutes of Health and Veterans Administration.

From the Department of Pathology and Laboratory Medicine, the Jewish Hospital of St. Louis; the Department of Pathology, Washington University School of Medicine, St. Louis; Veterans Administration Medical Center and Department of Microbiology, Vanderbilt University School of Medicine, Nashville, TN.

TWO DIFFERENT PATHWAYS OF INTERFERON MEDIATED SUPPRESSION OF ANTIBODY SECRETION

Interferon suppresses a variety of *in vitro* immune responses by a mechanism which has not been well defined. Both direct suppression and activation of suppressor T cells have been suggested as possible mechanisms of interferon action. In an attempt to examine this question, interferon-α (IFNα)-mediated suppression of a plaque forming cell response to a T cell independent antigen by spleen cells or by B cells was examined. Somewhat greater quantities of IFNα were required to suppress plaque forming cell responses by B cells than by spleen cells to the antigen fluoresceinated-*Brucella abortus* (FITC-BA). However, suppression of spleen cell responses could be blocked by addition of either 2-mercaptoethanol, levamisole or monoclonal antibodies against the lymphokine, soluble immune response suppressor (SIRS), whereas suppression of B cell responses by IFNα was unaffected by these agents. Each of these agents interferes with SIRS mediated suppression of immune responses. Addition of T cells to B cell cultures stimulated with FITC-BA did not affect the total plaque forming cell response nor the extent of suppression by IFNα, but it did restore 2-mercaptoethanol sensitivity to IFNα-mediated suppression. As few as 1 X 10^5 T cells were effective and it was necessary to add T cells within 3 h of addition of IFNα to confer 2-mercaptoethanol

sensitivity to IFNα-mediated suppression. These data suggest that IFNα can suppress immune responses by two different pathways and that in the presence of T cells, activation of suppressor T cells is the dominant pathway. The presence of T cells must also prevent direct suppression of B cells by IFNα.

Aune, T.M.

International Journal of Immunopharmacology 7(1):65-71, 1985.

Other support: Monsanto Company.

From the Department of Pathology and Laboratory Medicine, The Jewish Hospital of St. Louis, and the Department of Pathology, Washington University School of Medicine, St. Louis.

INHIBITION OF NATURAL KILLING BY ADENOSINE RIBONUCLEOTIDES

The effect of nucleotides on natural killer activity in mouse spleen was investigated. Adenosine ribonucleotides were found to strongly inhibit natural killer activity, with a half maximal inhibition at an ATP-concentration of 2.0 μM. The inhibition was highly specific for adenosine ribonucleotides; adenosine deoxyribonucleotides, guanosine deoxyribonucleotides, and guanosine ribonucleotides had very low, if any, inhibitory effect. Experiments with specific inhibitors showed that the effect is transmitted by purinergic receptor(s) located on the cell surface. Comparison of the kinetics of the ATP effect with the effects of trifluoperazine and quinacrine showed that ATP acts late in the lytic cycle, possibly immediately preceding the programming for lysis.

Henriksson, T. *(Blair, P.B.)*

Immunology Letters 7:171-176, 1983.

Other support: U. S. Public Health Service and the National Cancer Institute.

From the Department of Microbiology and Immunology, and the Cancer Research Laboratory, University of California, Berkeley.

ANTI-MY-26: A MONOCLONAL ANTIBODY INHIBITING HUMAN PHAGOCYTE CHEMILUMINESCENCE

Anti-My-26, a mouse monoclonal IgG1 antibody, was raised against human granulocytes and has been shown to inhibit luminol-enhanced, glucose-independent chemiluminescence (CL) of human granulocytes (or monocytes) responding to the soluble secretagogues A23187 or ionomycin (calcium ionophores) and phorbol myristate acetate (PMA). Anti-My-26 inhibition of CL was reversible and was dependent on both secretatogue and monoclonal antibody concentration. This inhibition appeared to be directed at the component of granulocyte CL that is independent of NAD(P)H-oxidase-catalyzed formation of superoxide anion, because neither opsonized zymosan-stimulated CL nor the PMA-induced decrease in NAD (P)H-associated autofluorescence was affected by anti-My-26. In addition, ionomycin, over a wide concentration range, failed to generate any decrease in granulocyte autofluorescence. The A32187-induced CL inhibited by anti-My-26 was correlated with its depression of oxygen consumption. Furthermore, anti-My-26 was not cytotoxic and did not itself

induce oxidative metabolism when used as a stimulant. Binding of anti-My-26 to phagocytic cells was not decreased by pre-exposure of cells to either A23187 or PMA. Evidence is presented to suggest that the binding of anti-My-26 to the granulocyte surface inhibits the oxidative response to calcium ionophore and PMA by blocking a common pathway(s) stimulated by these different secretagogues.

Warren, L. T. and *Civin, C.I.*

The Journal of Immunology 134(3):1827-1835, 1985.

Other support: National Institutes of Health.

From the Department of Biology, University of Pennsylvania, Philadelphia, and the Division of Pediatric Oncology, The Johns Hopkins Oncology Center, The Johns Hopkins University School of Medicine, Baltimore.

ANTI-MY-28, AN ANTIGRANULOCYTE MOUSE MONOCLONAL ANTIBODY, BINDS TO A SUGAR SEQUENCE IN LACTO-N-NEOTETRAOSE

Anti-My-28 is an IgM K monoclonal antibody produced by a hybridoma prepared from spleen cells of a mouse immunized with normal human granulocytes. By immunofluorescence it binds to human granulocytes but not to monocytes and lymphocytes. However, after treating cells with neuraminidase, the antibody also binds to lymphocytes and monocytes and to many leukemic cell lines and patient leukemic blast cells. Anti-My-28 binds to several neutral glycolipids and desialylated gangliosides of leukocytes and erythrocytes as shown by radioimmunoassay and immunostaining of thin-layer chromatograms. It recognizes a sugar sequence in lacto-N-neotetraose, Galβ1-4 GlcNAcβ1-3Galβ-4Glc. This tetrasaccharide occurs in the glycolipids paragloboside and sialosylparagloboside, and its distal trisaccharide sequence is found in higher glycolipids and glycoproteins.

Spitalnik, S. L., *Civin, C. I. et al.*

Blood 66(2):319-326, 1985.

Other support: National Institutes of Health.

From the National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases, Bethesda, MD, and the Division of Pediatric Oncology, the Oncology Center, The Johns Hopkins University School of Medicine, Baltimore, MD.

MIXED GLYCOSIDE PRETREATMENT REDUCES NONSPECIFIC BINDING OF ANTIBODIES TO FROZEN TISSUE SECTIONS

Indirect immunohistochemical studies of frozen mouse tissues with mouse monoclonal antibodies yield, in general, suboptimal results primarily because of indiscriminate binding of secondary antibody to all mouse immunoglobulins, i.e., to the monoclonal reagent and to endogenous immunoglobulin nonspecifically trapped in the tissue. To reduce this nonspecific staining, frozen sections of mouse kidney were treated enzymatically. Optimal results were obtained following a 2 hr treatment with 20 mg/ml of mixed glycosidases (MG). This treatment reduced the nonspecific background staining of the interstitial spaces and blood vessels, but did not affect the reactivity of structurally bound immunoglobulin G (IgG) in the glomeruli or alter the reactivity of

mouse renal tissue to the monoclonal antibody that recognizes an oligosaccharide antigenic determinant. Eluates from enzyme-treated frozen tissue sections contained normally immunoreactive IgG in the form of dimers. These data indicate that MG treatment of frozen sections could be safely used to reduce the content of nonstructurally bound immunoglobulins in frozen tissues and thus improve the visualization of specific monoclonal antibody binding.

Andrews, L. P., Clark, R. K., and *Damjanov, I.*

The Journal of Histochemistry and Cytochemistry **33**(7):695-698, 1985.

Other support: National Institutes of Health.

From the Department of Pathology and Laboratory Medicine, Hahnemann University School of Medicine, Philadelphia.

LOCALIZATION OF cAMP IN DOG AND CAT TRACHEA: EFFECTS OF β-ADRENERGIC AGONISTS

Adenosine 3',5'-cyclic monophosphate (cAMP) is believed to mediate the transport of ions, water, and mucous glycoproteins in the respiratory tract. Because chemical measurements of total tissue levels of cAMP may not always reflect changes in specific cell types, we adapted standard immunocytochemical methods to examine the cellular localization of cAMP in dog and cat tracheae. The β-adrenergic agonists terbutaline and isoproterenol increased immunoreactive cAMP in ciliated epithelial cells of dog and cat tracheae and in both serous and mucous gland cells of cat tracheae. Epithelial goblet cells did not respond to β-adrenergic agonists in either species. This study provides information about the location of β-receptors on individual cells in the trachea that is not available from chemical assays of either cAMP or β-receptors in these tissues. Our results support the hypothesis that secretory functions in both serous and mucous submucosal gland cells and ciliated epithelial cells, but not goblet cells, may involve cyclic AMP-dependent mechanisms.

Lazarus, S. C., Basbaum, C. B. and *Gold, W. M.*

American Journal of Physiology **247** (Cell Physiology 16):C327-C334, 1984.

Other support: National Heart, Lung and Blood Institute, and the Strobel Medical Research Fund of the American Lung Association of San Francisco.

From the Cardiovascular Research Institute and Departments of Medicine and Anatomy, University of California, San Francisco.

IMMUNOLOGIC RELEASE OF HISTAMINE FROM DOG LUNG: COMPARISON OF IN VIVO AND IN VITRO RESPONSES IN THE SAME ANIMAL

The purpose of this study was to compare, for the first time, antigen-induced histamine release from the lung in the same natively allergic dogs both *in vitro* and *in vivo*. In six dogs, maximal antigen-induced histamine release from the lung correlated closely *in vitro* and *in vivo* (r=0.94), although it varied widely between dogs (0% to 75.5% of total tissue histamine content); similarly, the antigen concentration to produce 50% of maximal histamine release varied sixfold between dogs (40 μg/ml to 250 μg/ml). In each

of five other dogs, terbutaline sulfate administered intravenously caused a dose-dependent inhibition of antigen-induced histamine release from lung fragments *in vitro*: the maximal inhibition produced by 1 mg/kg was 60 ± 4.5% (mean ± SEM). In these same dogs, 10^{-6}M terbutaline incubated with lung fragments *in vitro* caused inhibition of antigen-induced histamine release comparable to 1 mg/kg terbutaline *in vivo*. Increasing the dose of terbutaline *in vitro* produced maximal inhibition at 10^{-4}M with no greater effect of the drug at 10^{-3}M (71.4±3.8% inhibition). In both experimental situations propranolol caused a dose-dependent inhibition of β-adrenergic modulation of *Ascaris*-induced release of histamine. This result supports the conclusion that terbutaline produced its effects by actions mediated by β-adrenergic receptors on pulmonary mast cells. This experimental approach provides a suitable preparation in which to estimate the effective dose of agonists that modulate antigen-induced mast cell function *in vivo*.

Frey, M. J., Chesrown, S. E., Reed, B. R., Greenspon, L. W., Shields, R. L., Bourne, H. R., Brown, J. K., and *Gold, W. M.*

Clinical Immunology 74(5):728-729, 1984.

Other support: U. S. Public Health Service Pulmonary Specialized Center of Research.

From the Cardiovascular Research Institute and the Division of Clinical Pharmacology, Department of Medicine, University of California, San Francisco.

EXTRACELLULAR MATRIX MICROFIBRILS ARE COMPOSED OF CORE PROTEINS COATED WITH FIBRONECTIN

Extracellular proteins of cultured calf aortic smooth muscle cells consist predominantly of microfibrils 10-20 nm in diameter typical of "elastin-associated" microfibrils described in many tissues. Chemical and immunochemical evidence is presented that microfibrils consist of at least two proteins: core protein and fibronectin. Insoluble proteins of the microfibrils were obtained in the form of a pellet and antibodies raised in rabbits against these components. The antisera reacted with the insoluble microfibrillar proteins and with soluble fibronectin in enzyme-linked immunosorbent assay, and immunostained the extracellular microfibrils in cultured cells. An immunoglobulin (Ig) fraction was prepared and absorbed with fibronectin. The absorbed IgG retained its reactivity with the microfibrillar proteins but was no longer reactive with soluble fibronectin. Immunofluorescence studies were carried out using the absorbed IgG and IgG to soluble fibronectin. Both antibodies showed immunoreactive microfibrils in the extracellular matrix of cells in log phage. However, with increasing time in culture, as the cells reached confluence, the immunofluorescence of microfibrils reacting with the absorbed IgG became less intense, whereas that of microfibrils reacting with IgG to fibronectin increased; in confluent cells, essentially no staining was detected with the absorbed IgG, and a dense network of intensely stained microfibrils was seen with IgG to fibronectin. Treatment of these cultures with urea led to partial dissociation of the fibronectin and increased visualization of the microfibrils with the absorbed IgG; double-label immunofluorescence showed that both proteins occurred on the same microfibrils. The localization of immunoreactive sites to the extracellular microfibrils was confirmed by immunoelectron microscopy. Nearly quantitative cleavage with CNBr failed to dissociate the antigenically active fragments of fibronectin from the CNBr fragments of the core proteins of the microfibrils. It was concluded that microfibrils contain core proteins and fibronectin that are codistributed in insoluble, possibly cova-

lently cross-linked, aggregates. The core proteins are first deposited by the cell and, as a function of time in culture, fibronectin gradually coats their surface.

Schwartz, E., *Goldfischer, S.*, Coltoff-Schiller, B., and Blumenfeld, O. O.

The Journal of Histochemistry and Cytochemistry 33(4):268-274, 1985.

Other support: National Institutes of Health and the David Opochinsky-Henry Segal and the Blumenfeld Family Memorial Funds.

From the Departments of Biochemistry and Pathology, Albert Einstein College of Medicine, The Bronx, NY.

IMMUNOLOGIC INHIBITION OF ULTRAVIOLET RADIATION-INDUCED TUMOR SUPPRESSOR CELL ACTIVITY.

Long-term exposure of C3H mice to ultraviolet radiation resulted in the formation of suppressor T cells that recognize ultraviolet radiation-induced regressor skin cancers as a class before the appearance of overt tumors. Administration of monoclonal antibodies to the product of the I-J^k subregion of the major histocompatibility complex or low doses of cyclophosphamide in vivo inhibited the development or activity of these cells. This activity of the monoclonal antibody was eliminated by adsorption on B10.BR (I-J^k) but not B10.D2 (I-J^d) splenocytes. These findings provide evidence that elements expressing the I-J determinant are important in regulating the host response prior to the overt development of ultraviolet radiation-induced skin cancers and suggest novel therapeutic approaches to malignancies or other diseases involving suppressor T cells in their pathogenesis.

Granstein, R. D., Parrish, J. A., McAuliffe, D. J., Waltenbaugh, C., and *Greene, M. I.*

Science 224:615-617, 1984.

Other support: National Research Service Award and Arthur O. and Gullan M. Wellman Foundation.

From the Departments of Dermatology and Pathology, Harvard Medical School and Massachusetts General Hospital, Boston, and Department of Microbiology and Immunology, Northwestern University Medical School, Chicago.

EPIDERMAL ANTIGEN-PRESENTING CELLS IN ACTIVATION OF SUPPRESSION: IDENTIFICATION OF A NEW FUNCTIONAL TYPE OF ULTRAVIOLET RADIATION-RESISTANT EPIDERMAL CELL

In recent years, evidence has accumulated for the presence of immunologically active elements resident in the skin, which has led to the concept of skin-associated lymphoid tissues (SALT). Immunologic functions of these elements have been demonstrated to include processing and presentation of antigen to lymphocytes by dendritic cells called epidermal Langerhans cells (LC). Additionally, epidermal cell-derived, thymocyte-activating factor (ETAF), an entity biochemically and functionally very similar or identical to interleukin 1 (IL1), is produced by keratinocytes. Thus, the skin can be considered a complex organ in which cells relevant to the immune system are represented

Exposure of mice to low doses of ultraviolet radiation (UVR) *in vivo* leads to an inability to sensitize them to contact sensitizing reagents at the site of irradiation, and this depressed sensitization is accompanied by the formation of antigen-specific T suppressor (Ts) cells. It has also been demonstrated that hapten-coupled epidermal cells (EC) from UVR-treated mice are unable to immunize mice efficiently when administered subcutaneously, and such immunization results in the appearance of suppressor cells. We report that the murine epidermis contains a previously unrecognized antigen-presenting cell (APC) that is required for the activation of suppression, and that this APC is resistant to UVR. The dose of UVR employed is, however, sufficient to prevent substantial positive immunization of mice with syngeneic UV-irradiated hapten-coupled EC. These data explain in part the changes induced in epidermal antigen-presenting function by UVR, and has consequences for the understanding of UVR-induced cutaneous carcinogenesis.

Granstein, R. D., Lowy, A., and *Greene, M. I.*

The Journal of Immunology 132(2):563-565, 1985.

Other support: National Eye Institute, National Institutes of Health and Arthur O. and Gullan M. Wellman Foundation.

From the Departments of Dermatology and Pathology, Harvard Medical School, and Massachusetts General Hospital, Boston.

SPLENIC I-J BEARING ANTIGEN-PRESENTING CELLS IN ACTIVATION OF SUPPRESSION: FURTHER CHARACTERIZATION

A set of I-J-bearing murine splenic antigen-presenting cells (APC) has been found to be responsible for first order suppressor cell (Ts1, afferent suppressor cell) activation in the azobenzenearsonate (ABA) hapten system after intravenous administration. Suppressor cells induced by this set of hapten-coupled cells do not function in the efferent phase of the delayed hypersensitivity (DTH) response. The functional activity of this novel APC to activate afferent suppressor cells was resistant to a dose of ultraviolet radiation (UVR) sufficient to largely abrogate the ability of splenic APC to immunize for a DTH response. It was also found that the previously described splenic I-J-bearing APC needed for third-order suppressor cell (Ts3, effector-suppressor cell) activation is adherent and UVR resistant. The sets of I-J-bearing APC appear to be crucial elements in the activation of suppression and thus in determining the balance between immunologic reactivity and unresponsiveness. Furthermore, the UVR resistance of this set of novel APC may be relevant to the *in vivo* effects of UVR exposure to mice.

Granstein, R. D. and *Greene, M. I.*

Cellular Immunology 91:12-20, 1985.

Other support: Arthur O. and Gullan M. Wellman Foundation.

From the Departments of Dermatology and Pathology, Harvard Medical School and Massachusetts General Hospital, Boston.

MONOCLONAL IDIOTOPE VACCINE AGAINST *STREPTOCOCCUS PNEUMONIAE* INFECTION

A monoclonal anti-idiotope antibody coupled to a carrier protein was used to immunize BALB/c mice against a lethal *Streptococcus pneumoniae* infection. Vaccinated mice developed a high titer of antibody to phosphorylcholine, which is known to protect against infection with *Streptococcus pneumoniae*. Measurement of the median lethal dose of the bacteria indicated that anti-idiotope immunization significantly increased the resistance of BALB/c mice to the bacterial challenge. Antibody to an idiotope can thus be used as an antigen substitute for the induction of protective immunity.

McNamara, M. K., Ward, R. E. and *Kohler, H.*

Science 226:1325-1326, 1984.

Other support: U.S. Public Health Service and the New York State Department of Health AIDS Institute.

From the Department of Molecular Immunology, Roswell Park Memorial Institute, Buffalo, NY.

ANALYSIS OF A $T_{H1} \rightarrow T_{H2}$ HELPER CELL CIRCUIT

In the present study, the T15 idiotype-recognizing T helper cell circuit was dissected with respect to its homeostasis, interactive specificity, stability over time and effects on B cell expression. Analysis of the T_{H1} cells by adoptive transfer experiments indicates their short-lived state of activity during which T_{H2} cells are stimulated. T_{H1} cell activity was also directly monitored by the use of TNP-anti-T15 hybridoma antigens. It was found that T_{H1} cells are detected 1 wk after priming with PC-Hy, whereas T_{H2} cells become activated after 4 wk of priming. Comparative analysis of T_{H1} cells by using two different TNP-anti-T15 hybridoma antigens indicates a T_{H1} specificity for a shared idiotope. The stability over time of the $T_{H1} \rightarrow T_{H2}$ circuit was demonstrated by comparing T_{H2} frequencies in young and old mice.

Finally, we addressed the question of the function of the idiotype-recognizing T helper cells and showed that stimulation of T15-idiotype-specific T_{H2} cells can be correlated with a significant increase in the percentage of T15 idiotype in an anti-PC response. Collectively, these data describe an idiotype-specific T helper circuit as part of the network homeostasis of the immune system.

McNamara, M., Kang, C.-Y., and *Kohler, H.*

The Journal of Immunology 135(3):1603-1609, 1985.

Other support: National Institute on Aging.

From the Department of Molecular Immunology, Roswell Park Memorial Institute, Buffalo, NY.

IMMUNOREACTIVE CALCITONIN GENE-RELATED PEPTIDE AND SUBSTANCE P COEXIST IN SENSORY NEURONS TO THE SPINAL CORD AND INTERACT IN SPINAL BEHAVIORAL RESPONSES OF THE RAT

Using immunohistochemistry, evidence was obtained for the coexistence of calcitonin gene-related peptide (CGRP)- and substance P (SP)-like immunoreactivity in spinal sensory neurons. Analysis of caudally directed biting and scratching (CBS) behavior was carried out after intrathecal administration of CGRP and SP alone or in combination. Thus, SP (up to 20 μg) alone caused CBS only for a few minutes after injection, whereas SP (10 μg) plus CGRP (20 μg) caused a response with a duration up to 40 min. CGRP (20 μg) alone had no effects in this model. These findings provide support for a possible interaction of the two peptides at synapses in the dorsal horn of the spinal cord.

Wiesenfeld-Hallin, Z., Hokfelt, T., *Lundberg, J. M.*, Forssmann, W. G., Reinecke, M., Tshopp, F. A., and Fischer, J. A.

Neuroscience Letters 52:199-204, 1984.

Other support: The Swedish Medical Research Council, the Folksam Insurance Company, the Karolinska Institute, and the Swiss National Science Foundation.

From the Department of Clinical Neurophysiology, Huddinge Hospital; Departments of Histology and Pharmacology, Karolinska Institute, Stockholm; Department of Anatomy III, University of Heidelberg, Federal Republic of Germany; and the Research Laboratory for Calcium Metabolism, Departments of Orthopedic Surgery and Medicine, University of Zurich, Switzerland.

BIOSYNTHESIS OF GLYCOSPHINGOLIPIDS BY HUMAN MYELOID LEUKEMIA CELLS

We have performed comparative studies of the neutral glycosphingolipids synthesized by three human myeloid leukemia cell lines, K562, KG1, and HL-60, which were metabolically labeled with [^{14}C]galactose, to evaluate changes in neutral glycosphingolipid synthesis with myeloid cell differentiation. Individual neutral glycosphingolipids containing one to four sugars were purified by a combination of the following methods: diethylaminoethyl-Sephadex column chromatography, acetylation-Florisil column chromatography, and high-performance liquid chromatography using an Iatrobead column. Compounds with one sugar were analyzed by thin-layer chromatography on borate plates. This analysis showed that HL-60 cells synthesize only glucosylceramide, whereas K562 and KG1 cells synthesize predominantly [illegible]osylceramide, but also a small amount of galactosylceramide. Compounds with two to four s[illegible] were characterized by treatment with exo- and endoglycosidases. The results showed that K562 and KG1 cells are similar to cells from patients with acute leukemia in expressing two series (globo and neolacto) of natural glycosphingolipids, whereas the HL-60 cells are similar to mature human myeloid cells in expressing only one series (neolacto). Therefore, human myeloid leukemia cells blocked at different stages of differentiation vary in their ability to synthesize neutral glycosphingolipids.

Buehler, J., Qwan, E., DeGregoria, M. W., and *Macher, B. A.*

Biochemistry 24:6978-6984, 1985.

Other support: The Louis R. Lurie Foundation and the National Cancer Institute.

From the Cancer Research Institute and Department of Pharmaceutical Chemistry, University of California, San Francisco; Children's Cancer Research Institute, Pacific Presbyterian Medical Center, San Francisco.

HUMAN NATURAL ANTI-α-GALACTOSYL IgG II. THE SPECIFIC RECOGNITION OF α(1 →)-LINKED GALACTOSE RESIDUES

A natural IgG antibody (anti-Gal) with α-galactosyl binding specificity has been found in large amounts (0.5-1.0% of serum IgG) in all individuals tested. It has been purified by affinity chromatography on a column of melibiose-Sepharose. In addition to its affinity for normal and pathological senescent human red cells, the antibody readily interacts with rabbit red blood cell (RRBC) glycolipids with α-galactosyl terminal residues. Two types (glycosidic linkages of 1 → 3 *vs.* 1 → 4) of rabbit red cells glycolipids with terminal α-galactosyl residues were tested for antibody binding. The antibody specifically bound to glycolipids with Galα1 → 3 terminal residues, and treatment of these glycolipids with α-galactosidase abolished binding. Hemagglutination inhibition studies with oligosaccharides of known structure also showed that the antibody binds specifically to glycoconjugates with an α1 → 3 terminal galactose residue. Anti Gal did not bind to a human B-active glycolipid, indicating that fucose-linked α1 → 2 to the penultimate galactose prevents anti-Gal binding. The anti-Gal specificity for RRBC glycolipids also paralleled that of the α-galactosyl-specific *Bandeiraea simplicifolia* lectin. The possible reasons for the occurrence of this unique antibody in human serum are discussed.

Galili, U., *Macher, B. A.*, Buehler, J., and Shohet, S. B.

Journal of Experimental Medicine 162:573-582, 1985.

Other Support: National Institutes of Health.

From the MacMillan-Cargill Hematology Research Laboratory, Cancer Research Institute, Departments of Pharmaceutical Chemistry and Laboratory Medicine, University of California, San Francisco.

GLYCOSPHINGOLIPID CARRIERS OF CARBOHYDRATE ANTIGENS OF HUMAN MYELOID CELLS RECOGNIZED BY MONOCLONAL ANTIBODIES

Six monoclonal antibodies with known specificities for the carbohydrate antigens i, X or Y, and several anti-myeloid antibodies (determinants unknown) selected for their differing reaction patterns with human leucocytes were tested in chromatogram binding assays for reactions with myeloid cell glycolipids derived from normal human granulocytes and chronic myelogenous leukemia cells. Antigenicities were found exclusively on minor glycolipids which were barely or not at all detectable with orcinol-sulphuric acid stain. Among these, a neutral glycosphingolipid bound the anti-i antibody Den and chromatographed as the ceramide octasaccharide, (Galβ1 → 4GlcNAcβ1 → 3Galβ1 → 4GlcNAcβ1 → 3Galβ1 → 4GlcNAcβ1 → 3Galβ1 → 4Glc-Cer. Several species of neutral glycosphingolipids with six to more than ten monosaccharides were detected that carry the X antigen and others the Y antigen: Galβ1 → 4(Fucα1 → 3)GlcNAc and Fucα1 → 2Galβ1 → 4(Fucα1 → 3)GlcNAc, respectively. In addition, three new types of

carbohydrate specificities were detected among the myeloid cell glycolipids. Two were associated with neutral glycolipids: the first, recognized by anti-myeloid antibodies VIM-1 and VIM-10, was expressed on a distinct set of glycolipids with six or more monosaccharides, and the second, recognized by VIM-8, was expressed on glycolipids with more than ten monosaccharides. The third specificity, recognized by the anti-myeloid antibody VIM-2, was expressed on slow migrating sialoglycolipids with backbone structures of the poly-N-acetyllactosamine type that are susceptible to degradation with endo-β-galacosidase. Thus, we conclude that the i and Y antigens occur among the glycolipids of normal myeloid and chronic myelogenous leukemia cells and that a high proportion of hybridoma antibodies raised against differentiation antigens of myeloid cells are directed at carbohydrate structures.

Uemura, K., *Macher, B. A.*, DeGregorio, M., Scudder, P., Buehler, J., Knapp, W., and Feizi, T.

Biochimica et Biophysica Acta 846:26-36, 1985.

Other support: National Cancer Institute, North Atlantic Treaty Organization and Louis R. Lurie Foundation.

From the Applied Immunochemistry Research Group, Division of Communicable Diseases; Medical Research Council's Clinical Research Center, Middlesex (U.K.); Cancer Research Institute, Department of Pharmaceutical Chemistry, University of California, San Francisco; Children's Cancer Research Institute; Pacific Medical Center; and Department of Internal Medicine, Institute of Immunology, University of Vienna, Austria.

IN VITRO T CELL-MEDIATED KILLING OF *PSEUDOMONAS AERUGINOSA* I. EVIDENCE THAT A LYMPHOKINE MEDIATES KILLING

Previous studies have demonstrated *in vivo* that T cells can provide protective immunity, in the absence of antibody, against infection with the extracellular Gram-negative bacterium Immunotype 1 (IT-1) *Pseudomonas aeruginosa*. We established an *in vitro* system in which immune T cells, after reexposure to bacterial antigens and to macrophages, secrete a product that kills the bacteria. Although macrophages are required for *in vitro* killing, they function neither as antigen-presenting nor as phagocytic cells in this system. T cells from animals immunized against a different *P. aeruginosa* immunotype will not kill IT-1 organisms, but the supernatants produced by IT-1 immune T cells after exposure to macrophages and IT-1 *P. aeruginosa* organisms are nonspecifically effective in killing unrelated bacteria. Because the supernatants from immune T cells lose their bactericidal properties upon minimal dilution, we conclude that if this mechanism is active *in vivo*, it must play a role in local immunity.

Markham, R. B., Goellner, J., and Pier, G. B.

The Journal of Immunology 133(2):962-968, 1984.

Other support: Auxiliary of the Jewish Hospital of St. Louis; U.S. Public Health Service; National Institutes of Health; and U. S. Army Research and Development Command.

From the Departments of Medicine, and Microbiology & Immunology, Washington University School of Medicine, and The Jewish Hospital of St. Louis; Channing Laboratory, Harvard Medical School, and Brigham and Women's Hospital, Boston.

MEMBRANE AND CYTOSKELETAL CHANGES ASSOCIATED WITH IgE-MEDIATED SEROTONIN RELEASE FROM RAT BASOPHILIC LEUKEMIA CELLS

Binding of antigen to IgE-receptor complexes on the surface of RBL-2H3 rat basophilic leukemia cells is the first event leading to the release of cellular serotonin, histamine and other mediators of allergic, asthmatic and inflammatory responses. We have used dinitrophenol-conjugated bovine serum albumin (DNP-BSA) as well as the fluorescent antigen, DNP-B-phycoerythrin, and the electron-dense antigen, DNP-BSA-gold, to investigate dynamic membrane and cytoskeletal events associated with the release of [^{3}H]serotonin from anti-DNP-IgE-primed RBL-2H3 cells. These multivalent antigens bind rapidly to cell surface IgE-receptor complexes. Their distribution is initially uniform, but within 2 min DNP-BSA-gold is found in coated pits and is subsequently internalized. Antigen internalization occurs in the presence and absence of extracellular Ca^{2+}. The F-actin content of the detergent-extracted cell matrices analyzed by SDS PAGE decreases during the first 10–30 s of antigen binding and then increases by 1 min to almost double the control levels. A rapid and sustained increase is also observed when total F-actin is quantified by flow cytometry after binding of rhodamine-phalloidin. The antigen-stimulated increase in F-actin coincides with (and may cause) the transformation of the cell surface from a finely microvillous to a highly folded or plicated topography. Other early membrane responses include increased cell spreading and a 2-3-fold increase in the uptake of fluorescein-dextran by fluid pinocytosis. The surface and F-actin changes show the same dependence on DNP-protein concentration as stimulated [^{3}H] serotonin release; and both the membrane responses and the release of mediators are terminated by the addition of the non-cross-linking monovalent ligand, DNP-lysine. These data indicate that the same antigen-stimulated transduction pathway controls both the membrane/cytoskeletal and secretory events. However, the membrane and actin responses to IgE-receptor-cross-linking are independent of extracellular Ca^{2+} and are mimicked by phorbol myristate acetate, whereas ligand-dependent mediator release depends on extracellular Ca^{2+} and is mimicked by the Ca^{2+} ionophore A23187.

Pfeiffer, J. R., Seagrave, J. C., Davis, B. H., Deanin, G. D., and *Oliver, J. M.*

The Journal of Cell Biology 101:2145-2155, 1985.

Other support: American Cancer Society and National Institutes of Health.

From the Department of Pathology, University of New Mexico School of Medicine, Albuquerque, and the Department of Pathology, State University of New York, Upstate Medical Center, Syracuse.

DISTRIBUTION OF GALANIN IMMUNOREACTIVITY IN THE RESPIRATORY TRACT OF PIG, GUINEA PIG, RAT, AND DOG

Galanin, a newly discovered peptide isolated from porcine intestine, is known to cause contraction in rat smooth muscle preparations and to induce hyperglycemia in dogs. By the use of radioimmunoassay and immunohistochemical techniques the concentration and distribution of galanin immunoreactivity were determined in several areas of the respiratory tract of five dogs, five guinea pigs, five rats, and two pigs. Antibodies were raised in rabbits to whole unconjugated natural porcine galanin. The highest galanin concentrations were found in the bronchus and the trachea of the dog,

guinea pig, rat (2 pmol/g in each case), and pig (≤ 1 pmol/g). The lowest galanin concentrations were found in the lung parenchyma. Gel chromatographic analysis in the pig showed one molecular form of galanin coeluting with the porcine galanin standard. By means of the indirect immunofluorescence technique on sections of tissues fixed in benzoquinone solution, galanin was found to be confined to nerve fibres in different regions of the respiratory tract. In the nasal mucosa of the pig, nerve fibers containing galanin were distributed around seromucus glands and blood vessels and beneath the epithelium. In the trachea, bronchus, and major intrapulmonary airway of the pig, dog, and guinea pig, galanin immunoreactive fibers were detected predominantly in smooth muscle, as well as around seromucus glands and in the adventitia of blood vessels. Rarely, galanin immunoreactive nerve fibers were found in the lung parenchyma. A few galanin immunoreactive ganglion cells also containing vasoactive intestinal polypeptide were found in the adventititia of the tracheobronchial wall of the pig and dog. The distribution of galanin suggests that this peptide may have some influence on airway, vascular and secretory functions in the mammalian respiratory tract.

Cheung, A., *Polak, J. M.*, Bauer, F. E., Cadiux, A, Christofides, N. D., Springall, D. R., and Bloom, S. R.

Thorax 40:889-896, 1985.

Other support: Medical Research Council (United Kingdom).

From the Departments of Histochemistry and Medicine, Royal Postgraduate Medical School, London, England.

ANALYSIS OF MACROPHAGE DIFFERENTIATION AND FUNCTION WITH MONOCLONAL ANTIBODIES

A large number of anti-mouse and anti-human macrophage/monocyte monoclonal antibodies (MAb) have recently been obtained that are proving invaluable reagents of extraordinary specificity for the study of macrophage differentiation, function, and surface antigen structure. This chapter summarizes information on such MAb up to February 1983. In the mouse, at least five antigens that can be distinguished by molecular weight have been found on macrophages but not on lymphocytes. One of the mouse Fc receptors, present on macrophages as well as on some lymphocytes, has also been defined with MAb. Further antigens have not been defined biochemically but appear to have distinct distributions on functional subpopulations. Mac-1 was the first antigen to be defined by MAb that is present on macrophages and not on lymphocytes. A second antigen has been discovered that is distinct from Mac-1 in cell distribution, function, and α-subunit structure, but appears to use the same β-subunit. In the course of studies on the molecular basis of T-cell function, MAb were selected for their ability to inhibit antigen-specific T-lymphocyte-mediated killing. It appears that LFA-1 is distinct from the antigen receptor, but works together with it in contributing to the avidity of the CTL for the target cell. LFA-1 is present on B lymphocytes and myeloid cells as well as T lymphocytes, suggesting that it plays a more general role in adhesion than do antigen receptors. A similar family of related molecules has been found on human cells. Other sections of this study deal with MAC-2 antigen, Mac-3 antigen, Langerhans cells, dendritic cells, and macrophages, defining macrophages by their surface markers, and immunological evolution.

Springer, T. A. and Unkeless, J. D.

In: Adams, D. O. and Hanna, M. G., Jr. (eds.): *Contemporary Topics in Immunobiology* Vol. 13, New York: Plenum Publishing Corporation, 1984, pp. 1-31.

Other support: U. S. Public Health Service and the American Cancer Society.

From the Laboratory of Membrane Immunochemistry, Harvard Medicall School, Boston, and the Laboratory of Cellular Physiology and Immunology, The Rockefeller University, New York.

PREPARATION AND USE OF MONOCLONAL ANTIMACROPHAGE ANTIBODIES

Macrophages are a diverse family of cells originating from pluripotent stem cells in the bone marrow. Outside the marrow, macrophages can take the form of blood monocytes, Kupffer cells, alveolar macrophages, peritoneal macrophages, Langerhans cells, and in almost every organ, as "fixed tissue macrophages." In addition to variation in anatomical localizations, macrophages can exhibit heterogeneity in function and state of differentiation. The advent of hybridoma technology has allowed the identification of over 40 macrophage antigens, some of which are useful for distinguishing macrophages from other cells whereas others are associated with distinct subsets of macrophages. In this section, a number of monoclonal antibodies with relatively restricted specificities for macrophages were chosen and their characteristics were summarized. Possible applications of these antibodies are also described.

Ho, M.-K. and *Springer, T. A.*

Methods in Enzymology 108:313-325, 1984.

Other support: U. S. Public Health Service.

From Harvard Medical School, Boston.

THE LFA-1, Mac-1 GLYCOPROTEIN FAMILY AND ITS DEFICIENCY IN AN INHERITED DISEASE

A family of functionally important, high-molecular-weight glycoproteins with identical β subunits has recently been defined on leukocyte cell surfaces. Soon after these molecules and at least some of their functions had been defined with monoclonal antibodies, an inherited disease, LFA-1, Mac-1 deficiency, was discovered in humans. This deficiency has confirmed that this glycoprotein family is of central importance in leukocyte cell surface adhesion reactions.

Springer, T. A.

Federation Proceedings 44:2660-2663, 1985.

Other support: National Institutes of Health.

From the Dana-Farber Cancer Institute, Harvard Medical School, Boston.

THE SEVERE AND MODERATE PHENOTYPES OF HERITABLE Mac-1, LFA-L DEFICIENCY: THEIR QUANTITATIVE DEFINITION AND RELATION TO LEUKOCYTE DYSFUNCTION AND CLINICAL FEATURES.

An inherited syndrome characterized by recurrent or progressive necrotic soft-tissue infections, diminished pus formation, impaired wound healing, granulocytosis, and/or delayed umbilical cord severance was recognized in four male and four female patients. As shown with subunit-specific monoclonal antibodies in immunofluorescence flow cytometry and ^{125}I immunoprecipitation techniques, in addition to a tritiated sodium borohydride-galactose oxidase labeling assay, granulocytes, monocytes or lymphocytes from these individuals had a "moderate" or "severe" deficiency of Mac-1, LFA-1, or p150,95 (or a combination) - three structurally related "adhesive" surface glycoproteins. Two distinct phenotypes were defined on the basis of the quantity of antigen expressed. Three patients with severe deficiency and four patients with moderate deficiency expressed < 0.3% and 2.5-31% of normal amounts of these molecules on granulocyte surfaces, respectively. The severity of clinical infectious complications among these patients was directly related to the degree of glycoprotein deficiency. More profound abnormalities of tissue leukocyte mobilization, granulocyte-directed migration, hyperadherence, phagocytosis of iC3b-opsonized particles, and complement- or antibody-dependent cytotoxicity were found in individuals with severe, as compared with moderate, deficiency. It is proposed that *in vivo* abnormalities of leukocyte mobilization reflect the critical roles of Mac-1 glycoproteins in adhesive events required for endothelial margination and tissue exudation. The recognition of phenotypic variation among patients with Mac-1, LFA-1 deficiency may be important with respect to therapeutic strategies.

Anderson, D. C. *Springer, T. A. et al.*

The Journal of Infectious Diseases 152(4):668-689, 1985.

Other support: National Institute of Allergy and Infectious Diseases, National Institute for Cancer Research, National Institutes of Health, and U. S. Department of Agriculture.

From the Departments of Pediatrics, Microbiology and Immunology, and Pathology, Baylor College of Medicine, Houston, and Dana-Farber Cancer Institute, Harvard Medical School, Boston.

MACROPHAGE AND T LYMPHOCYTE-MEDIATED IMMUNITY: SIMILARITIES AT THE LEVEL OF THE MAC-1 AND LFA-1 MOLECULES

A number of cell surface molecules have recently been characterized which are important in macrophage and lymphocyte cell-mediated immunity. The macrophage Fc receptor and complement receptor type one (CR_1) have been characterized by purification and function-inhibiting antibodies. Recently, anti-Mac-1 antibody was found to inhibit the complement receptor type three (CR_3), suggesting that the CR_3 is identical to or associated with the previously biochemically characterized Mac-1 molecule. Studies on T lymphocyte-mediated immunity with function-blocking antibodies have shown that Lyt-2,3 and LFA-1 surface molecules are associated with T lymphocyte-mediated killing. Somewhat surprisingly, the Mac-1 molecule associated with CR_1 function, and the LFA-1 molecule associated with T-lymphocyte-mediated killing, have been found to be structurally related. This suggests that at least with regard

to these molecules, similar molecular mechanisms underlie macrophage and T lymphocyte-mediated immunity. The findings on Mac-1 and LFA-1 are reviewed and the evolutionary implications are discussed.

Springer, T. A.

In: Volkman, A. (ed.): *Mononuclear Phagocyte Biology*. New York: Marcel Dekker, 1984, pp. 109-128.

Other support: U. S. Public Health Service and an American Cancer Society Junior Faculty Award.

From Harvard Medical School, Boston.

FUNCTIONAL AND STRUCTURAL INTERRELATIONSHIPS AMONG THE Mac-1, LFA-1 FAMILY OF LEUKOCYTE ADHESION GLYCOPROTEINS, AND THEIR DEFICIENCY IN A NOVEL HERITABLE DISEASE

Cell surface adherence reactions are of central importance in the immune functions of lymphocytes, monocytes, and granulocytes. Lymphocytes adhere to antigen-presenting macrophages or dendritic cells in the induction of T-lymphocyte immune responses and to target cells in cell-mediated killing. Adhesive interactions are fundamental to a wide spectrum of functions of granulocytes, monocytes, and macrophages. Specific recognition of opsonized microorganisms is facilitated by membrane receptors for IgG and for the third component of complement (C3), which mediate microbe-cell adhesion prior to the triggering of cytoskeletal events leading to endocytosis. Adhesion mediated by IgG (Fc) receptors can also trigger antibody-dependent killing of target cells, independently of endocytosis. A family of high-molecular-weight glycoproteins with identical β subunits has recently been characterized on leukocyte surfaces that is important in many of the above adhesion reactions. Monoclonal antibodies have been instrumental in elucidating the structural interrelationships and functions of these three glycoproteins, macrophage antigen 1 (Mac-1), lymphocyte function-associated antigen 1 (LFA-1), and p150,95. Furthermore, this research has led to the definition, with monoclonal antibodies, of a novel heritable disease that manifests itself in defects in leukocyte adherence and motility. With the use of MAb, a novel disease has been recognized in which the Mac-1, LFA-1 and p150,95 glycoproteins are deficient. Recurrent bacterial infection, progressive periodontitis, persistent leukocytosis, and/or delayed umbilical cord separation have been described in patients whose neutrophils demonstrated depressed phagocytic function and deficient adherence and chemotaxis.

Springer, T. A. and Anderson, D. C.

In: Springer, T. A. (ed.): *Hybridoma Technology in the Biosciences and Medicine*, Chap. 11, New York: Plenum Publishing Coporation, 1985, pp. 101-206.

Other support: National Institutes of Health.

From the Laboratory of Membrane Immunochemistry, Dana-Farber Cancer Institute, Department of Pathology, Harvard Medical School, Boston, and Baylor College of Medicine, Department of Pediatrics, Houston, TX.

STUDIES ON ANTIGENS ASSOCIATED WITH THE ACTIVATION OF MURINE MONONUCLEAR PHAGOCYTES: KINETICS OF AND REQUIREMENTS FOR INDUCTION OF LYMPHOCYTE FUNCTION-ASSOCIATED (LFA)-1 ANTIGEN *IN VITRO*

Macrophages activated and primed *in vivo*, although not resident or responsive macrophages, express the lymphocyte function associated (LFA)-1 antigen. By contrast, the biochemically related Mac-1 antigen is expressed on all populations of macrophages. In the present paper, we studied regulation of the LFA-1 antigen in vitro. LFA-1 could be induced *in vitro* on thioglycollate (TG)-elicited but not on proteose peptone (PP)-elicited or resident macrophages. Specifically, macrophage-activating factor (MAF), interferon-γ (IFN-γ), or picogram amounts of endotoxin (LPS) induced LFA-1 on TG-elicited macrophages following overnight incubation. Interferon, -α or -β, fucoidin, and colony-stimulating factor were not effective. While some levels of LFA-1 could be detected as soon as 10 hr peak expression was observed after 16 to 32 hr of incubation. The induction could be completely abrogated by cycloheximide, suggesting that protein synthesis was required. These results indicate that the induction of LFA-1 on mononuclear phagocytes is closely regulated and that the requirements for such induction are distinct from, but share certain similarities with, induction of cytotoxic functions and expression of Ia antigen

Strassmann, G., *Springer, T. A.*, and Adams, D. O.

The Journal of Immunology 135(1):17-150, 1985.

From the Department of Pathology, Duke University Medical Center, Durham, NC, and the Laboratory of Membrane Immunochemistry, Dana-Farber Cancer Institute, Harvard Medical School, Boston.

COMPLEMENT RECEPTOR TYPE THREE-DEPENDENT DEGRADATION OF OPSONIZED ERYTHROCYTES BY MOUSE MACROPHAGES

The role of the complement receptor type 3 (CR3) on thioglycollate-elicited peritoneal macrophages (TG-PM) in the destruction of opsonized particles was studied. We found that sheep red blood cells (E) that were opsonized with an IgM monoclonal anti-Forssman antibody and complement (E-IgM-C) were lysed by TG-PM, whereas there was little lysis of E pretreated with either the antibody or the complement source alone. Furthermore, this lysis could be inhibited by anti-CR3 monoclonal antibodies that had previously been shown to inhibit binding of E-IgM-C to the CR3. Kinetic studies of phagocytosis and lysis indicated that lysis of E-IgM-C occurs after phagocytosis, suggesting that lysis is an intracellular event. Further findings suggested that intracellular lysis was promoted by CR3 bound to the phagocytosed target, because a monoclonal anti-CR3 antibody decreased the rate of phagocytosis of E-IgM-C but not its magnitude, whereas the rate and extent of lysis were strikingly inhibited. Furthermore, TG-PM that had already internalized unopsonized E selectively lysed E-IgM-C that were added later. These data confirm that the interaction of the CR3 with its ligand on E-IgM-C promotes rapid phagocytosis, and they further suggest that the CR3 facilitates degradation of the target particle once internalization has occurred.

Rothlein, R. and *Springer, T. A.*

The Journal of Immunology 135(4):2268-2672, 1985.

Other support: U.S. Public Health Service.

From the Laboratory of Membrane Immunochemistry, Dana-Farber Cancer Institute, Harvard Medical School, Boston.

THE FUNCTION OF LFA-1 IN CELL-MEDIATED KILLING AND ADHESION: STUDIES ON HERITABLE LFA-1, Mac-1 DEFICIENCY AND ON LYMPHOID CELL SELF-AGGREGATION

Lymphocyte function associated antigen-1 (LFA-1) is a cell surface glycoprotein identified in mouse and human by monoclonal antibodies which inhibit cytolytic T lymphocyte (CTL) mediated cytolysis. Recently, a number of patients with recurring, life-threatening infections were found to be deficient in LFA-1 and two other surface molecules which utilize the same β subunit, Mac-1 and p150,95. To assess natural killing, peripheral blood lymphocytes (PBL) from LFA-1 deficient individuals, their families, and unrelated controls were cultured alone or with JY cells for six days. Natural killing was assessed on the K562 erythroleukemia cell line (HLA negative). All LFA-1 deficient individuals showed low levels of NK cell-mediated cytolysis compared to that of family members and unrelated individuals. PBL were also tested for proliferation after stimulation with the lectin phytohemagglutinin (PHA). PBL from LFA-1 deficient patients showed an impaired proliferative response to PHA. Contrasting results were found for the quantitatively more severely LFA-1 deficient CTL of patients 1 and 2. Killing by patient 2 CTL was poorly inhibited when anti-LFA-1 MAb was added to the assay, and pretreatment of killers or effectors gave equivical or no blocking. CTL from patient 1 was inhibited when anti-LFA-1 was included in the assay. The findings with patient 1 and 4 CTL suggest that LFA-1 on target cells can also contribute to the CTL-target interaction. Data presented here suggest a strong simularity between phorbol ester-induced cell-cell aggregation and the LFA-1-dependent adhesion step in CTL-mediated killing, and suggest LFA-1 functions as an adhesion protein.

Springer, T. A. et al.

In: Henlart, P. and Martz, E. (eds.): *Mechanisms of Cell-Mediated Cytotoxicity II*. New York & London: Plenum Press, 1985, pp. 311-322.

Other support: National Institutes of Health.

From Dana-Farber Cancer Institute, Harvard Medical School, Boston; Baylor College of Medicine, Houston, TX; and Stanford University, Stanford, CA.

STRUCTURAL ORGANIZATION OF INTERPHASE 3T3 FIBROBLASTS STUDIED BY TOTAL INTERNAL REFLECTION FLUORESCENCE MICROSCOPY

We studied the laminar organization of 3T3 fibroblast cells growing on glass slides by use of total internal reflection illumination to excite fluorescence emission (TIRF) from labeled molecules and stained cellular compartments that are very close to the cell-substrate contact region. Mitochondria, distant from the contact regions and stained with the water-soluble cationic dye, dil-C_3-(3), fluoresced only as the glass/cytoplasm critical angle was approached. A similar result was obtained when the nuclei were stained with Hoechst dye 33342. From this measured angle a cytoplasmic refractive index in the range 1.358-1.374 was computed. The plasma membrane of 3T3 cells was stained with dil-C_{18}-(3) and the cytoplasmic compartment was stained with fluoresceinyl-dextran (FTC dextran) or with carboxyfluorescein. We have

demonstrated a high degree of correspondence between the low-reflectance zones in the reflection interference image of a live cell and the TIRF images of both the plasma membrane and cytoplasmic compartment. TIRF photometry of selected contact regions of cells provided data from which the absolute separation of cell and substrate was computed. From a population of 3T3 cells microinjected with fluorescein-labeled actin, motile and adherent interphase cells were selected for study. For adherent cells, which displayed fluorescent stress fibers, the TIRF image was composed of intense patches and less intense regions that corresponded, respectively, to the focal contact and close-contact zones of the reflection-interference image. The intense patches corresponded to the endpoints of the stress fibers. Cells of motile morphology, which formed some focal contacts and extensive close-contact zones, gave AF-actin TIRF images of relatively even intensity. Thin lamellar regions of the cytoplasm were found to contain concentrations of actin not significantly different from other close-contact regions of the cell. The major analytical problem of TIRF microscopy is separation of the effects of proximity to substrate, refractive index and fluorescent probe concentration on the local brightness of the TIRF image. From our results, it appears possible to use TIRF microscopy to measure the proximity of different components of substrate contact regions of cells.

Lanni, F., Waggoner, A. S., and *Taylor, D. L.*

The Journal of Cell Biology 100:1091-1102, 1985.

From the Center for Fluorescence Research in Biomedical Sciences and the Department of Biological Sciences, Carnegie-Mellon University, Pittsburgh.

LIGHT-SCATTERING CHANGES DURING CHEMOTACTIC STIMULATION OF HUMAN NEUTROPHILS: KINETICS FOLLOWED BY FLOW CYTOMETRY

The light-scattering properties of human neutrophils were compared on a cell-by-cell basis before and after stimulation with chemotactic peptide using flow cytometry. Between 20 and 180 sec after peptide addition, side (90°) scatter declined by up to 4% and forward scatter increased up to 6%. Between 3 and 15 min, side scatter increased up to 15% and forward scatter decreased up to 5%. Association of a fluorescence chemoattractant with neutrophils was most rapid during the initial phase of increasing forward and decreasing side scatter, and association saturated before the maximum increase in side scatter. Evidence is presented that the observed changes in scatter were not a consequence of chemoattractant-induced cell-cell adhesion or neutrophil degranulation. Rather, the early phases of light-scattering changes are interpreted to represent membrane ruffling by the stimulated neutrophil; the later phases polarization of the neutrophil morphology.

McNail, P. L., Kennedy, A. ., Waggoner, A. S., *Taylor, D. L.*, and Murphy, R. F.

Cytometry 6:7-12, 1985.

Other support: National Institutes of Health, National Science Foundation, and Health Research and Services Foundation.

From the Center for Fluorescence Research in Biomedical Sciences and the Department of Biological Sciences, Carnegie-Mellon University, Pittsburgh.

CONDITIONS REQUIRED FOR EXPRESSION OF MEMBRANE IL 1 ON B CELLS

The authors have reported that macrophages bear a mitogenic activity for T cells in their plasma membrane that we have termed "membrane-interleukin 1". Membrane IL 1 was studied by using either paraformaldehyde-fixed macrophages or isolated macrophage membranes. The reason for ascribing this membrane activity to IL 1 was based on the observation that macrophage membranes stimulated proliferation of thymocytes and an IL 1-dependent T cell line but not IL 2-dependent T cells, and this activity was inhibited by a polyclonal goat anti-IL 1 antibody. Additional biochemical studies need to be done to unequivocally establish the interrelationships between the soluble IL 1 and the membrane form. Nevertheless, two important features of the membrane IL 1 were noteworthy: first, it was essential that Ia-bearing, fixed macrophages express it to activate antigen-specific T cell lines, and second, its expression could be dissociated from the secretion of IL 1. Because B cells are known to function as antigen-presenting cells, we investigated whether these cells express membrane IL 1.

Kurt-Jones, E. A., Kiely, J. M. and *Unanue, E. R.*

The Journal of Immunology 135(3):1548-50, 1985.

Other support: National Institutes of Health.

From the Department of Pathology, Harvard Medical School, Boston.

IMMUNE COMPLEX EFFECT ON MURINE MACROPHAGES
I. IMMUNE COMPLEXES SUPPRESS INTERFERON-γ INDUCTION OF Ia EXPRESSION

We have studied the effects of immune complexes on the expression of macrophage surface proteins *in vitro*. Increased expression of the H-2 molecules I-A, I-E, and K on the macrophage membrane was induced by *in vitro* culture with crude lymphokine or interferon-γ. Expression of all three of the molecules was additional increased by stimulating the cultures with heat-killed *Listeria monocytogenes*. Addition of soluble immune complexes to the cultures did not have any effect on macrophage expression of these proteins. However, significant inhibition of lymphokine or interferon-γ induction of I-A, I-E, and H-2K was observed when macrophages were cultured on plates to which immune complexes had been bound. This inhibition was dose dependent, required an immunoglobulin (Ig) molecule with an intact Fc portion, did not require the presence of T cells, and occurred in the presence of indomethacin. Complexes containing IgG1, IgG2a, IgG2b, and IgE, but not IgM or IgA, antibodies mediated the inhibitory effect.

Virgin, H. W. IV, Wittenberg, G. F., and *Unanue, E. R.*

The Journal of Immunology 135(6):3735-3743, 1985.

Other support: National Institutes of Health and National Institute of General Medical Sciences.

From the Harvard Medical School, Department of Pathology, Boston.

IMMUNE COMPLEX EFFECTS ON MURINE MACROPHAGES.
II. IMMUNE COMPLEX EFFECTS ON ACTIVATED MACROPHAGES
CYTOTOXICITY, MEMBRANE IL-1, AND ANTIGEN PRESENTATION

We investigated the effects of immune complexes on macrophage functions *in vitro*. Immune complexes inhibit lymphokine induction of both I-A^k expression and cytotoxic activity by fetal calf serum elicited macrophages during long-term (7 days) culture. In addition, induction of antigen presentation was significantly inhibited by immune complexes. Expression of membrane interleukin 1 (IL-1, a membrane-bound form of the T cell mitogen required for antigen presentation by fixed cells) was minimally inhibited by immune complexes. Therefore, inhibition of antigen presentation was primarily due to effects on Ia expression rather than membrane IL-1 expression. The inhibitory effect of immune complexes was not found during short-term culture (4 to 48 hr) when activated macrophages (bearing high levels of Ia) from mice infected with *Listeria monocytogenes* were examined. Immune complexes maintained or even increased levels of both I-A^k and cytotoxicity in activated macrophages. The implications of these findings for immune complex modulation of the immune response are discussed.

Virgin, H. W. IV, Kurt-Jones, E. A., Wittenberg, G. F. and *Unanue, E. R.*

The Journal of Immunology 135(6):3744-3749, 1985.

Other support: National Institutes of Health; U. S. Public Health Service; National Institute of General Medical Sciences.

From Harvard Medical School, Department of Pathology, Boston, MA.

SUPPRESSION OF IMMUNE RESPONSE TO *Listeria monocytogenes*:
MECHANISM(S) OF IMMUNE COMPLEX SUPPRESSION

We have investigated possible mechanisms underlying immune complex suppression of resistance to *Listeria monocytogenes*. Inhibition of resistance was found when immune complexes were formed *in vivo* in immune mice or in nonimmune mice adoptively transferred with specific antibody. Suppression was also found when nonimmune mice were injected with immune complexes preformed *in vitro*. We investigated the role of complement by decomplementing mice with cobra venom factor purified by high-pressure liquid chromatography. Complete depletion of serum C3 did not eliminate immune complex suppression of resistance to *L. monocytogenes*, suggesting that complement activation is not required for immune complex suppression. Infection-induced changes in the surface phenotype and functional properties of macrophages from normal and immune complex-suppressed mice were also investigated. Macrophage expression of both H-2K and Ia molecules increased during the response of normal mice to *L. monocytogenes*. However, these changes were not found in immune complex-suppressed mice. In contrast, membrane interleukin 1 expression was increased in macrophages from suppressed mice compared with macrophages from normal mice. Macrophages from *L. monocytogenes*-infected normal and immune complex-suppressed mice expressed cytotoxicity against tumor cells *in vitro*. We conclude that immune complexes do not inhibit resistance to *L. monocytogenes* by activation of complement or decreasing macrophage cytoxic activity. Rather, defects in Ia expression by macrophages from suppressed mice might be one component responsible for immune complex suppression of resistance of *L. monocytogenes*.

Virgin, H. W. IV, Wittenberg, G. F., Bancroft, G. J., and *Unanue, E. R.*

Infection and Immunity 50(2):343-353, 1985.

Other support: U.S. Public Health Service, National Institutes of Health, and National Institute of General Medical Sciences.

From the Department of Pathology, Harvard Medical School, Boston.

RELATIONSHIP OF MACROPHAGE Ia AND MEMBRANE IL 1 EXPRESSION TO ANTIGEN PRESENTATION

Membrane expression of Ia molecules by antigen-presenting cells is critical for the induction of T cell responses to foreign protein antigens. In addition, antigen-specific T cell proliferation has been thought to be dependent on interleukin 1 (IL 1) secretion by antigen-presenting cells. Recently, we have described a novel membrane-bound form of IL 1 that is required for the presentation of antigen by antigen-pulsed, fixed macrophages. Membrane IL 1 is a mitogenic protein found on the macrophage membrane that, like soluble IL 1, stimulates thymocytes and IL 1-dependent T cells but not IL 2-dependent T cell lines. This activity is inhibited by a polyclonal anti-IL 1 antibody. Membrane IL 1 is an integral membrane protein and does not represent soluble IL 1 nonspecifically bound or fixed to the macrophage membrane. Additional biochemical studies are required to define the possible interrelationships between soluble and membrane IL 1.

We have now developed a method for independently varying the levels of Ia and membrane IL 1 expression on macrophages in culture. By then fixing the cells, we are able to preserve constant levels of these molecules during the assay for antigen presentation. Using this system, we have demonstrated that quantitative variation in these two parameters was associated with changes in the magnitude of the T cell response.

Kurt-Jones, E. A., Virgin, H. W. IV, and *Unanue, E. R.*

The Journal of Immunology 135(6):3652-3654, 1985.

Other support: National Institutes of Health.

From the Department of Pathology, Harvard Medical School, Boston.

ACUTE LUNG INJURY IN RAT CAUSED BY IMMUNOGLOBULIN A IMMUNE COMPLEXES

Mouse IgG and IgA, with reactivity to dinitrophenol conjugated to carrier protein, have been isolated from myeloma proteins by means of a variety of affinity techniques. The IgA was predominantly in the dimeric form. The *in vitro* and *in vivo* biological activities of IgA-containing immune complexes were assessed in the rat.

IgA-containing immune complexes were demonstrated, in a dose-dependent manner *in vitro*, to activate neutrophils and to generate O_2^-. In addition, these immune complexes showed evidence of complement activation *in vitro*, by the use of immunofixation techniques. When IgA was instilled into the airways of rats and antigen was injected intravenously, acute lung injury occurred, as reflected by increases in lung permeability and morphological changes consisting of blebbing of endothelial cells, intra-alveolar hemorrhage, and fibrin deposition. The lung changes were directly proportional to the amount of IgA instilled into the airways and failed to occur if intravenous injection of antigen was omitttedi Lung injury did not occur in animals that received an intravenous injection of antigen in the absence of an airway injection of

IgA. Lung injury related to IgA-containing immune complexes was complement dependent but neutrophil independent. In companion studies with mouse IgG-containing immune complexes, acute lung injury also occurred and had morphological features similar to those associated with IgA-induced lung injury except that, in the case of IgG immune complex-induced damage, neutrophils were more evident. Acute lung injury induced by IgG-containing immune complexes, whether of mouse or rabbit origin, was complement and neutrophil dependent. The similarities and differences between IgG- and IgA-associated acute immune complex-induced injury of rat lung were reinforced by the use of morphometry techniques.

Studies with another monoclonal IgA antibody-containing, antigen-binding activity to phosphorylcholine also demonstrated the ability of IgA antibody to cause acute lung injury in the rat. Neither antibody alone nor antigen (phosphorylcholine linked to bovine serum albumin) alone produced evidence of lung injury.

These studies indicate for the first time that immune complexes containing IgA have lung-damaging properties and that the pathogenic mechanisms are different from those associated with IgG-associated immune complex-induced acute lung injury.

Johnson, K. J., Wilson, B. S., Till, G. O., and *Ward, P. A.*

Journal of Clinical Investigations 74:358-369, 1984.

Other support: National Institutes of Health.

From the Department of Pathology, University of Michigan Medical School, Ann Arbor.

THE DNA SYNTHETIC RESPONSE OF NORMAL AND ABNORMAL HUMAN LYMPHOCYTES TO MEVALONIC ACID: THE ROLE OF GRANULOCYTES AS A HELPER POPULATION

In order to investigate the role of neutrophils in the DNA synthetic response of human peripheral blood lymphocytes to mevalonic acid, we obtained preparations of both cells each free of cross-contamination by the other. Purified lymphocytes respond poorly to mevalonic acid, but their response can be significantly enhanced by one-half their number of neutrophils. Preincubation of lymphocytes with neutrophils for 24 hr, even in the absence of mevalonic acid, further increases the lymphocyte response. We have been unable to demonstrate the production by granulocytes of either an intracellular or extracellular mevalonate-derived growth factor that in turn stimulates lymphocytes. Granulocytes preexposed to mevalonate do not acquire the ability to stimulate lymphocyte DNA synthesis in the absence of mevalonate. Our experiments suggest that neither neutrophil lysosomal enzymes nor reactive oxygen species generated by neutrophils are responsible for the help neutrophils provide. Normal E rosette-positive lymphocytes fail to respond to mevalonate, whereas E rosette-negative cells do. The mevalonate response of normal E rosette-negative cells is enhanced by the presence of granulocytes in contrast to B cell-chronic lymphocytic leukemia cells that synthesize DNA briskly in response to mevalonic acid in the absence of neutrophil help. These observations add to our knowledge of the relationship between mevalonate metabolism and the regulation of cellular DNA synthesis and mitosis.

Larson, R. A., Kluskens, L. E. and *Yachnin, S.*

Journal of Allergy and Clinical Immunology 74:280-291, 1984.

Other support: National Institute of Arthritis, Metabolism and Digestive Diseases and Nalco Cancer Research Fund.

From the Departments of Medicine and Pathology and the Committee on Immunology, The University of Chicago School of Medicine.

OXYGENATED CHOLESTEROLS SYNERGISTICALLY IMMOBILIZE ACYL CHAINS AND ENHANCE PROTEIN HELICAL STRUCTURE IN HUMAN ERYTHROCYTE MEMBRANES

Fourier transform infrared spectroscopy revealed that insertion of 20α-hydroxycholesterol into human erythrocyte membranes (10% of total membrane sterol) immobilized the lipid acyl chains to a degree equivalent to enriching total membrane cholesterol by 50%. Raman spectroscopy showed that the amount of acyl chain rotamers was not significantly altered by the presence of 20α-hydroxycholesterol, indicating that acyl chain immobilization was limited to an inhibition of lateral motion. The presence of 20α-hydroxycholesterol may synergistically enhance the acyl-chain-immobilizing behavior of membrane cholesterol. In addition, protein helical structure was not altered by 20α-hydroxycholesterol. The insertion of 7α-hydroxycholesterol into erythrocyte membranes resulted in an increase in protein helical structure which was comparable to that observed for erythrocyte membranes enriched with pure cholesterol by 50%. However, both acyl chain mobility and conformation were unchanged. These results suggest a synergistic behavior between oxysterols and cholesterol in modifying erythrocyte membrane packing.

Rooney, M. W., *Yachnin, S.*, Kucuk, O., Lis, L. J., and Kauffman, J. W.

Biochimica et Biophysica Acta 820:33-39, 1985.

Other support: Northwestern University Research and U.S. Public Health Service.

From the Biomedical Engineering Division, Northwestern University, Technological Institute, Evanston, IL; the Department of Medicine, the Pritzker School of Medicine, University of Chicago; the Department of Medicine, the Chicago Medical School; and the Department of Physics and the Liquid Crystal Institute, Kent State University, OH.

VII. Metabolic Studies

EXPOSURE OF SMALL AIRWAYS TO CRISTOBALITE IN VITRO

In the studies reported here, tissue explants from different levels of rodent airways, from trachea to bronchioles as small as 200 microns in diameter, were exposed to nontoxic concentrations of cristobalite particles of a size less than 20 microns. The authors were interested in the effects of cristobalite, a known toxic and fibrinogenic particulate, upon mucin secretion and production of prostaglandins and leukotrienes by the exposed epithelial cells. They hypothesized that exposure to cristobalite could affect metabolism of arachidonic acid in these cells. Depending on the enzymatic pathways affected, this could lead to increased synthesis of products of the cyclooxygenase cascade that could inhibit mucin secretion (PGE) or stimulatory products of the lipoxygenase pathway (leukotrienes C and D). As was seen, the results of these studies suggest cristobalite has a differential effect on mucin secretion by explants of rodent airway tissue from different levels of the respiratory tree. As one progresses deeper into the intrapulmonary airways and into the small bronchioles, slight stimulstion of secretion changes to significant inhibition.

Adler, K. B. et al.

In: NATO ASI Series, Vol. G3, Beck, E. G. and Bignon, J. (eds.) *In Vitro Effects of Mineral Dust*. Berlin Heidelberg: Springer-Verlag, 1985.

Other support: National Institutes of Health.

From the Departments of Pathology, Civil Engineering and Physiology/Biophysics, University of Vermont, Burlington.

CYTOCHALASIN D-INDUCED INCREASE IN ACTIN SYNTHESIS AND CONTENT IN A VARIETY OF CELL TYPES

Treatment of a variety of mesenchymal cells (normal and transformed rat fibroblasts, bovine aortic endothelial cells, rabbit smooth muscle cells) exhibiting different cytoskeletal organizations and derived from several species with doses of cytochalasin D (CD, 2-6 μM for 20 h) sufficient to induce cytoskeletal rearrangement and altered cellular morphology results in an increase in the relative content and rate of synthesis of actin. These data extend our previous findings for HEp-2 cells to other cell types and provide further evidence for our hypothesis that the CD-induced cytoskeletal reorganization triggers stimulation of actin synthesis and the resulting increase in actin content.

Brett, J. G., Tannenbuam, J. and *Godman, G. C.*

Cell Biology International Reports 9(8):723-730, 1985.

Other support: National Science Foundation and National Institutes of Health.

From the the Department of Pathology, College of Physicians & Surgeons of Columbia University, New York.

MEMBRANE CYCLING AND MACROVACUOLATION UNDER THE INFLUENCE OF CYTOCHALASIN: KINETIC AND MORPHOMETRIC STUDIES

Fibroblasts exposed to higher doses of cytochalasin accumulate very big, discrete, endoplasmic vacuoles, the membrane of which is derived by internalization of plasmalemma. Morphometry confirms that the amount of surface interiorized is equal to the difference betwen the original cell surface area (before CD) and the reduced surface area measurable after CD-induced rounding. Correspondingly, there is a nearly two-fold increase in the activity of the ectoenzyme 5'-nucleotidase (a marker for plasma membrane) internally within the cytoplasm, after treatment with CD. Macrovacuolation increases cell volume by ~ 30%. Surface membrane is internalized as micropinocytotic vesicles at a rate measurable by the accumulation of horseradish peroxidase (HRP), a marker of fluid-phase pinocytosis. Uptake of HRP is shown to be enhanced at all times during exposure to CD and is balanced by accelerated exocytic recycling of membrane except during a phase (~ 4-8 hr) in which pinocytic uptake exceeds exocytosis. Vesicular membrane accumulated intracellularly in this period is retained in the endoplasm and by successive fusions forms vacuoles in close approximation to microfilament aggregates. Once established, this new macrovacuolar membrane compartment is in dynamic equilibrium with the cell surface and its membrane is cycled like the plasma membrane, in a mutual exchange of pinosomes between the several vacuoles and the cell surface. In drug-free medium, vacuole membrane apparently reverts to the surface by pinocytotic recycling, and the cells recover normal characteristics 4-6 hr after withdrawal of cytochalasin.

Brett, J. G. and *Godman, G. C.*

Tissue & Cell 16(3):325-335, 1984.

Other support: National Institutes of Health.

From the Department of Pathology, College of Physicians & Surgeons of Columbia University, New York.

PEROXISOMAL DEFECTS IN NEONATAL-ONSET AND X-LINKED ADRENOLEUKODYSTROPHIES

Accumulation of very long chain fatty acids in X-linked and neonatal forms of adrenoleukodystrophy (ALD) appears to be a consequence of deficient oxidation of very long chain fatty acids, a function that has been attributed to peroxisomes. Peroxisomes were readily identified in liver biopsies taken from a patient having the X-linked disorder. However, in liver biopsies from a patient having neonatal-onset ALD, hepatocellular peroxisomes were greatly reduced in size and number, and sedimentable catalase was markedly diminished. The presence of increased concentrations of serum pipecolic acid and the bile acid intermediate, trihydroxycoprostanic acid, in the neonatal ALD patient are associated with a generalized diminution of peroxisomal activities that was not observed in the patient with X-linked ALD.

Goldfischer, S. et al.

Science 227:67-70, 1985.

Other support: National Institutes of Health, National Science Foundation and Gail I. Zuckerman Foundation.

From the Departments of Pathology, Pediatrics, and Neurology and the Liver Research Center, Albert Einstein College of Medicine, The Bronx, NY; the Departments of Pathology and Pediatric Neurology, Children's Hospital of Michigan, and Wayne State University School of Medicine, Detroit; the Departments of Cell Biology and Medicine and Kaplan Cancer Center, New York University School of Medicine, New York; the John F. Kennedy Institute, the Johns Hopkins University School of Medicine, Baltimore, MD; and Rockefeller University, New York.

ULTRASTRUCTURAL AND CYTOCHEMICAL DEMONSTRATION OF PEROXISOMES IN CULTURED FIBROBLASTS FROM PATIENTS WITH PEROXISOMAL DEFICIENCY DISORDERS

The oxidation of very long chain fatty acids and synthesis of ether glycerolipids (plasmalogens) occurs mainly in peroxisomes. Zellweger's cerebrohepatorenal syndrome (CHRS) is a rare, inherited metabolic disease characterized by an apparent absence of peroxisomes, an accumulation of very long chain fatty acids, and a decrease of plasmalogens in tissues and cultured fibroblasts from these patients. As peroxisomes are ubiquitous in mammalian cells, we examined normal and CHRS-cultured fibroblasts for their presence, using an electron microscopic histochemical procedure for the subcellular localization of catalase, a peroxisomal marker enzyme. Small (0.08-0.20 μm) round or slightly oval peroxisomes were seen in both normal and CHRS fibroblasts. The number of peroxisomes was analyzed morphometrically and found to be significantly reduced in all CHRS cell lines. These results are discussed in relation to the underlying defect in peroxisomal function and biogenesis in this disease.

Arias, J. A., Moser, A. B., and *Goldfischer, S. L.*

The Journal of Cell Biology 100:1789-1792, 1985.

Other support: National Institutes of Health.

From the Albert Einstein College of Medicine, The Bronx, NY, and The Johns Hopkins School of Medicine, Baltimore, MD.

PROCESSING OF ANGIOTENSIN AND OTHER PEPTIDES BY THE LUNGS

The pulmonary vascular bed can be considered as a sluice gate for controlling the quality of biologically active peptides allowed to enter the systemic circulation. The remarkable feature of the lung is the selectivity of the processing, especially when one considers that most peptides are indiscriminately degraded by blood enzymes, tissue homogenates, and many enzymes. One of the most critical determinants of the selectivity of metabolism of peptides is the location of their inactivating enzymes. Thus it is the cell biology that to a great extent determines the access of peptides to their metabolizing enzymes. The present chapter is devoted to the processing of polypeptide hormones by the lungs. Bradykinin and angiotensin (ANG) are the most studied of the physiologically important peptide hormones processed by the lungs. In the discussion section of this paper, it is noted that the biological activities of the kinins and ANG II disappear, whereas the activity of ANG I is enhanced during circulation through various vascular beds. To a large degree the changes in activities of the vasoactive polypeptides are independent of enzymes and cellular elements of blood but are closely related to peptidase activities of the vascular bed itself. Over the past several years, aspects of the metabolism of kinins and ANG I by pulmonary endothelium have been defined. The

focus has been on a cell not previously known to exist until the advent of electron microscopy. Endothelial cells selectively process a series of vasoactive substances, including ANG I and bradykinin. Stidues of the pulmonary processing of angiotensins and related peptides clearly show that the critical, initial reactions occur at the cell surface — this is true of metabolic enzymes, receptors, and the release of products. A challenge for the future is to begin to chart the geography of the pulmonary endothelial cell surface.

Ryan, U. S.

In: Fishman, A. P. and Fisher, A. B. (eds.): *Handbook of Physiology - The Respiratory System I*, Bethesda, MD: The American Physiological Society, 1985, Chap. 10, pp. 351-364.

Other support: National Institutes of Health.

From the Department of Medicine, University of Miami School of Medicine, Miami, FL.

THE PULMONARY ENDOTHELIAL SURFACE

The understanding of endothelial metabolic properties has increased dramatically in recent years. Endothelial cells (ECs) possess hormones, drugs, and many blood-borne substances by means of enzymes and transport processes. In turn, some hormones, blood cells, and cellular products interact with ECs via specific receptors on the luminal surface. Functional complexity is exemplified by the metabolism of the adenine nucleotides. ATP, ADP, and AMP are metabolized by enzymes of the endothelial surface to release adenosine, which may be immediately taken up into endothelium and reincorporated intracellularly into nucleotides. Equally complex is the metabolism of the kinins and angiotensins by ECs. Bradykinin is inactivated whereas angiotensin I is converted to angiotensin II. Bradykinin not thus degraded can act on endothelial receptors and stimulate the release of prostacyclin (PGI_2). Thus, bradykinin can amplify the release of another vasodilator, PGI_2, and can stimulate the release of a powerful antiaggregatory agent (PGI_2). Many of these complex metabolic reactions occur at the endothelial surface, a structure that is itself complex. ECs possess endothelial projections and caveolae as well as a fuzzy coat, or glycocalyx. Functions of the endothelial glycocalyx are not well understood, but the glycocalyx cannot be visualized; it may act as a molecular sieve and provide a substratum for the initiation and progression of immunologic reactions.

Ryan, U. S., Ryan, J. W. and Crutchley, D. J.

Federation Proceedings 44:0013-0019, 1985.

Other support: National Institutes of Health.

From the Department of Medicine, University of Miami School of Medicine, Miami, FL, and the Research Division, Miami Heart Institute, Miami Beach, FL.

PULMONARY ENDOTHELIUM AND PROCESSING OF PLASMA SOLUTES: STRUCTURE AND FUNCTION

While the lungs often are considered exclusively in terms of gas exchange, they have additional functions not necessarily related to such exchange. One such activity is the ability to process selectively hormones, hormone precursors, and other excitatory substances as they pass through the lungs via the bloodstream. As the lungs convert venous blood to arterial blood, they also regulate the entry of hormonal substances into the systemic arterial circulation. Through this selective processing, products of specific reactions of the lungs can influence specific actions of tissues and organs at a distance. The first level of compartmentation in the lungs is the separation of the blood supply from the air supply. Quite clearly, circulating substances are unlikely to have access to enzymes beyond the first cellular layer lining the vessels — the endothelium. The present chapter describes functional and structural aspects of the interaction of solutes and colloids of plasma with pulmonary endothelial cells. First the basic architecture and environment of the lungs are considered. The structure and situation of the lungs within the circulatory system which make them well suited for gas exchange may well explain how the lungs are so efficient in processing some hormones. The next level of compartmentation is within the pulmonary circulation and is governed by the flow characteristics and relative surface areas of the vessels. Figures are given in this section which emphasize the overwhelming significance of endothelial cells of the pulmonary microvasculation in providing a surface for interaction with blood-borne substrates. In following sections of this paper, Immunocytochemistry, Endothelial Cell Culture and Surface Specializations (Endothelial Projections, Caveolae, and Glycocalyx), are discussed. It will be important for future studies to examine to what extent modulation of endothelial surface structure — projections, caveolae, glycocalyx, enzyme receptors, and transport molecules — affect the overall functioning of endothelium as a tissue and the overall functioning of the lungs in maintaining the quality of the internal milieu.

Ryan, U. S. and Frøkjaer-Jensen, J.

In: Said, S. I. (ed.): *The Pulmonary Circulation and Acute Lung Injury*. Mount Kisco, NY: Futura Publishing Co., Inc., 1985, pp. 37-60.

Other support: National Institutes of Health.

From the University of Miami School of Medicine, Miami, FL.

EVIDENCE FOR A ROLE OF HYDROXYL RADICAL IN IMMUNE-COMPLEX-INDUCED VASCULITIS

Previously it was shown that tissue injury occurring in acute immune-complex-induced vasculitis, which is complement- and neutrophil-dependent, is significantly attenuated by the presence of catalase, suggesting the pathogenic role of H_2O_2 generated from activated neutrophils. We now show that significant protection is also afforded by pretreatment of animals with apolactoferrin, a naturally occurring chelator of iron. Iron-saturated lactoferrin is devoid of protective effects. Deferoxamine mesylate, a synthetic iron chelator, also has protective effects. Infusion of ionic iron, especially Fe(III), potentiates the tissue injury. Significant protection from tissue injury is also produced by treatment of rats with dimethyl sulfoxide, a potent hydroxyl radical scavenger. Morphologically, animals treated with these protective interventions show the influx of neutrophils into sites of immune complex deposition, but there is markedly attenuated edema, little or no hemorrhage, and little evidence of

endothelial cell injury, in contrast to the findings in nonprotected animals. These data support the suggestion that immune-complex-induced injury may be linked to generation of H_2O_2 from activated neutrophils and the subsequent conversion of peroxide to the hydroxyl radical.

Fligiel, S. E. G., *Ward, P. A.*, Johnson, K. J., and Till, G. O.

American Journal of Pathology 115:375-382, 1984.

Other support: National Institutes of Health.

From the Department of Pathology, the University of Michigan Medical School, Ann Arbor.

EVIDENCE FOR THE ROLE OF OXYGEN RADICALS IN ACUTE NEPHROTOXIC NEPHRITIS

Acute glomerular injury in the rat has been induced by the intrarenal, intraarterial infusion of sheep antibody to glomerular basement membrane anti-GBM (antiglomerular basement membrane). The antiglomerular basement membrane antibody has been verified to be of the variety that is complement and neutrophil dependent for the induction of acute proteinuria, which peaks during the first 24 hours. Following injection of the antibody, acute, intense, glomerular injury resulted, with the denuding of glomerular vascular basement membrane associated with extensive damage or destruction of glomerular endothelial cells and fusion of epithelial cell foot processes. Treatment of animals with catalase produced, in a dose-dependent manner, as much as 75% protection against glomerular injury, as assessed by reduction in the proteinuria. Treatment of animals with superoxide dismutase caused a small reduction in the degree of glomerular injury, again assessed by a reduction in proteinuria. However, this protective effect of superoxide dismutase was not found to be statistically significant. The hydroxyl radical scavenger, dimethyl sulfoxide, which has been shown to protect against endothelial cell injury following systemic activation of complement, was not protective in the anti-GBM model. Morphologically, glomeruli from catalase-protected rats showed numerous neutrophils but little or no evidence of injury of either glomerular endothelial or epithelial cells. These data suggest that acute glomerular injury produced by antiglomerular basement membrane is related to H_2O_2 production from activated neutrophils.

Rehan, A., Johnson, K. J., Wiggins, R. C., Kunkel, R. G., and *Ward, P. A.*

Laboratory Investigation 51(4):396-402, 1984.

Other support: National Institutes of Health.

From the Departments of Internal Medicine and Pathology, the University of Michigan Medical School, Ann Arbor.

RAT NEUTROPHIL ACTIVATION AND EFFECT OF LYPOXYGENASE AND CYCLOOXYGENASE INHIBITORS

Activation (defined as lysosomal enzyme secretion and generation of O_2^- of rat neutrophils has been measured with the use of varying doses of soluble stimuli (phorbol myristate acetate (PMA); calcium ionophore A23187; and N-formyl-methionyl-leucyl-phenyl-alanine (FMLP) and particulate agents (immune complexes

and zymosan particles). With either the calcium ionophore or the chemotactic peptide (FMLP), substantial enzyme release occurred, but the amount of O_2^- produced was very small. Cytochalasin B greatly enhanced the enzyme release response to the chemotactic peptide but had little effect on neutrophil responses to other soluble stimuli. The cell response to PMA resulted in the greatest production of O_2^- with significant enzyme secretion. When cell stimulation with insoluble stimuli (immune complexes or zymosan particles) was studied, significant amounts of enzyme release occurred in parallel with the generation of substantial amounts of O_2^-. The presence of cytochalasin B enhanced the cell responses to immune complexes but had an inhibitory effect on zymosan-induced responses. As expected, the amount of lysozyme secreted by stimulated rat neutrophils tended to exceed the amount of β-glucuronidase released from the same cells.

Neutrophil responses were investigated in the presence of drugs that were demonstrated in the rat neutrophil to inhibit either the lipoxygenase of the cyclooxygenase pathways. Inhibitors of the cyclooxygenase pathway (indomethacin, piroxicam, ibuprofen, BW755C), with few exceptions, consistently enhanced the enzyme secretion response, while effects on O_2^- generation were less clear-cut but tended to be predominantly inhibitory. Drugs with inhibitory effects on the lipoxygenase pathway (nordihydroguaiaretic acid and nafazatrom) had significant inhibitory effects on both enzyme secretion as well as generation of O_2^-. These data suggest that activation responses (enzyme secretion and O_2^- generation) of rat neutrophils may be dissociated (*i.e.*, one not always accompanying the other). Further, it appears that neutrophil activation, as defined by enzyme secretion, is enhanced by products of the lipoxygenase pathway and suppressed by products of the cyclooxygenase pathway. Generation of O_2^- is not affected in such a clear-cut manner. Taken together, the data suggest that enzyme release and O_2^- production by activated rat neutrophils may be under separate control.

Ward, P. A., Sulavik, M. C., and Johmson, K. J.

American Journal of Pathology 116:223-233, 1984.

Other support: National Institutes of Health.

From the Department of Pathology, the University of Michigan Medical School, Ann Arbor.

DIFFERENTIATION OF A HUMAN LEUKEMIA CELL LINE AND EXPRESSION OF COLLAGENASE INHIBITOR

A human collagenase inhibitor (CI) of M_r 29,500 has been extensively characterized in skin fibroblasts and identified in a variety of connective tissues. Because human alveolar macrophages synthesize and secrete both a collagenase and CI that are immunologically and functionally identical to their counterparts in fibroblasts, we studied the production of such proteins by an immature human cell line (HL60) that can be induced to differentiate along monocytic or granulocytic pathways. The cells failed to synthesize collagenase under any culture condition tested. However, upon exposure to 1,25-dihydroxyvitamin D_3 or phorbol esters (PMA), both of which promote monocytic differentiation of HL60, these cells synthesized and released CI in a dose-dependent manner. Furthermore, the extent of CI expression was paralleled by the acquisition by such cells of the monocytic marker 63D3, indicating that inhibitor production and differentiation are closely correlated. This CI was immunologically and functionally

identical to that produced by human macrophages and human skin fibroblasts. The quantity of CI synthesized by PMA-stimulated cells was 3- to 5-fold greater than produced by human alveolar macrophages, ≈ 1 μg per 10^6 cells per day. In contrast, undifferentiated HL60 cells produced little or no detectable CI (≤ 10-20 ng per 10^6 cells per day). Interestingly, when HL60 cells were stimulated to undergo granulocytic differentiation by dimethyl sulfoxide or retinoic acid, they also produced the "monocytic" CI.

Shavit, Z. B., *Welgus, H. G. et al.*

Proceedings of the National Academy of Sciences of the United States of America 82:5380-5384, 1985.

Other support: National Institutes of Health.

From the Division of Cell Biology, Washington University School of Medicine; Division of Dermatology, Department of Medicine, The Jewish Hospital at Washington University Medical Center, St. Louis; and Division of Dermatology, Department of Medicine, VA Medical Center/University of Tennessee Center for the Health Sciences, Memphis.

FAMILIAL AGGREGATION IN CHRONIC OBSTRUCTIVE PULMONARY DISEASE: USE OF THE LOGLINEAR MODEL TO ANALYZE INTERMEDIATE ENVIRONMENTAL AND GENETIC RISK FACTORS

To examine the contribution of environmental and genetic risk factors to familial aggregation in chronic obstructive pulmonary disease (COPD), 325 first-degree (1d) relatives and 56 spouses of 150 COPD patients were compared with 222 1d relatives and 49 spouses of 107 nonpulmonary patient controls for the prevalence of two clinical outcomes: 1) airways obstruction (AO; 1-sec forced expiratory volume ≤ 68% of forced vital capacity) and 2) chronic bronchitis (CB; cough and sputum for 3+ months per year for 2+ years). The loglinear model was used to study direct and indirect (*i.e.*, those mediated by other risk factors) components of familial aggregation. Three risk factors were found to be independently associated with CB and/or AO: α_1-antitrypsin deficiency (PiZ allele), personal cigarette smoking, and parental cigarette smoking. Because 1d relatives of COPD patients were more likely to have a PiZ allele, be heavy smokers (1+ packs per day), and be exposed to parental smoking than 1d relatives of controls, these three factors also constituted indirect components of familial aggregation. However, after controlling for the three factors, 1d relatives of COPD patients were more likely to have AO and CB than 1d relatives of controls (direct component). This direct component might have a genetic basis because no such association was found when spouses instead of 1d relatives were compared. Thus, both shared environmental factors (personal and passive smoking) and shared genetic factors (α_1-antitrypsin and a possible direct genetic component) contribute to familial aggregation in COPD. The loglinear model provides a useful tool for analyzing familial aggregation in diseases of multifactorial etiology.

Khoury, M. J., Beaty, T. H., Tockman, M. S., Self, S. G., and *Cohen, B. H.*

Genetic Epidemiology 2:155-166, 1985.

Other support: Lebanese National Council for Scientific Research and National Institutes of Health.

From the Departments of Epidemiology, Biostatistics and Environmental Health Sciences, The Johns Hopkins University School of Hygiene and Public Health, Baltimore.

HEALTH RELATED PSYCHOSOCIAL CORRELATES OF NEUROTICISM: A STUDY OF ADULT MALE TWINS IN FINLAND

Some health related psychosocial correlates of the Eysenck neuroticism scale were examined in a questionnaire study of 1001 monozygotic (MZ) and 3455 dizygotic (DZ) male twin pairs representing the adult male twin population in Finland. In analyses of the individuals, 34% of the variance in neuroticism was associated with psychological variables (stress of daily activities, life satisfaction, quality of sleep, and extroversion—the explanatory rate of this variable set was 30%), psychotropic drugs (5%), alcohol use (4%), and smoking (2%). Neuroticism was also associated with social, life change, and medical variables. In pairwise analyses, the heritability estimate (h^2) was 0.54 for pairs living together and 0.39 for pairs living apart. It seems that heritability

estimates are confounded by the closer intrapair relationship between members of MZ than DZ pairs. In pairwise analyses, 23% of the intrapair difference of neuroticism in MZ pairs was associated with intrapair differences in the aforementioned variables. The following explananatory rates were found: psychological variables, 21%; psychotropic drugs, 2%; alcohol use, 2%; and smoking, 1%. Neuroticism of pairs discordant for background variables showed similar intrapair differences as between individuals in the following variables: service *vs.* farming work, use of alcohol, use of antacids, hypertension, heavy physical work, quality of sleep, changes of workplace for negative reasons, smoking, and use of tranquillizers. It appears that in Finland, environmental factors explain at least 61% of the variability in neuroticism, and that factors determining neuroticism are also associated with health related behavior such as smoking, use of alcohol and psychotropic drugs.

Koskenvuo, M., Langinvainio, H., Kaprio, J., and Sarna, S.

Acta Geneticae Medicae et Gemellologiae 33:307-320, 1984.

From the Department of Public Health Science, University of Helsinki, Helsinki, Finland.

FINNISH TWINS REARED APART II. VALIDATION OF ZYGOSITY, ENVIRONMENTAL DISSIMILARITY AND WEIGHT AND HEIGHT

Within the Finnish Twin Cohort of like-sexed adult twin pairs, a subgroup of pairs separated at an early age has been identified. In 165 pairs, both cotwins responded to questionaires in 1975 and 1979. An environmental dissimilarity score was formed which consists of items on whether the twins had lived after separation in the same community, attended the same school, were in the same grade at school, how often they met, how often they met common friends and relatives, and whether they attended the same clubs or not, *etc.* To validate the zygosity diagnosis obtained by questionnaire in 1975, those pairs whose zygosity was unknown and those with the least contact after separation were contacted for blood sampling (11 bloodgroups). Of 15 pairs with no zygosity diagnosis, 10 responded (1 no address, 2 abroad, 2 refused). Six pairs were classified MZ and 4 DZ. In 12 MZ and 8 DZ pairs undergoing bloodgroup determination, the classification of only one pair changed from DZ to MZ. The following intraclass correlations for height and weight were found:

Age at	No. Cases		Weight		Height	
separation	MZ	DZ	MZ	DZ	MZ	DZ
0-5	18	61	0.88	0.31	0.88	0.70
0-10	30	95	0.87	0.36	0.92	0.70

Langinvainio, H., *Koskenvuo, M.*, Kaprio, J., and Sistonen, P.

Acta Geneticae Medicae et Gemellologiae 33:251-258, 1984.

Other support: Yrjo Jahnsson Foundation.

From the Department of Public Health Science, University of Helsinki, and Finnish Red Cross Blood Transfusion Service, Helsinki, Finland.

FINNISH TWINS REARED APART III. PERSONALITY FACTORS

This study is based on data from 165 adult twin pairs separated at 10 years of age or less. Information on personality factors: extraversion (E) and neuroticism (N) (EPI scale short form), life satisfaction (LS) (Allardt) and stress of daily activities (SDA) was obtained as part of the questionnaire study carried out in the entire Finnish Twin Cohort in 1975. Later in 1979 a questionnaire sent to the twins reared apart yielded a scale (range 7-30 points) measuring the environmental dissimilarities after separation (reliability 0.83). The effect of separation on personality factors by analysis of variance of individual data was studied. The overall explanatory rates were low (2.1 - 4.4%). The definitive study group was formed by selecting those pairs with a dissimilarity score greater than 15. The following intraclass correlations were obtained:

Age at separation	No.of cases		E		N		LS		SDA	
	MZ	DZ	MZ	DZ	MZ	DZ	MZ	DZ	MZ	DZ
0.5	18	61	0.40	0.17	0.34	0.07	0.22	0.18	0.04	0.00
0-10	30	95	0.38	0.12	0.25	0.11	0.40	0.19	0.06	0.02

Langinvainio, H., Kaprio, J., *Koskenvuo, M.*, and Lonnqvist, J.

Acta Geneticae Medicae et Gemellologiae 33:259-264, 1984.

Other support: Yrjo Jahnsson Foundation

From the Departments of Public Health Science and Psychiatry, University of Helsinki, Finland.

PSYCHIATRIC HOSPITALIZATION IN TWINS

Hospitalization rates of monozygotic (MZ) and dizygotic (DZ) twin pairs in Finland were compared for schizophrenia, neuroses, and alcoholism. Record-linkage of hospital records and death certificates for the years 1972-1979 was carried out for persons in the Finnish Twin Cohort (16,649 like-sexed twin pairs). The ratio of the number of observed *vs.* that of expected concordant pairs and the ratio of concordance rates between MZ and DZ pairs were greater among males than females, and greater among young (40 years old or less) than among older pairs. The highest difference was found in schizophrenia and the lowest in neuroses. Pairwise concordance rates for schizophrenia (11.0% for MZ and 1.8% for DZ) seem to indicate great environmental influence (high proportion of discordant pairs) with apparent genetic liability (6.1-fold ratio in concordance between MA and DZ pairs). In neurotic disorders, the difference of pairwise concordance rates between MZ and DZ pairs (6.6% vs. 4.0%) was quite low, not strongly supporting a genetic hypothesis. Of the MZ pairs concordant for psychiatric hospitalization, 47% had lived together for their whole life time; of those discordant, 16% lived together. The corresponding figures for DZ pairs were 18% and 15%. The effect of intrapair relationships in disease-concordant pairs should be taken into account when evaluating the effect of genetic and environmental factors in psychiatric disorders.

Koskenvuo, M., Langinvainio, H., Kapirio, J., Lonnqvist, J., and Tienari, P.

Acta Geneticae Medicae et Gemellologiae 33:321-332, 1984.

From the Department of Public Health Science and Department of Psychiatry, University of Helsinki, and Department of Psychiatry, University of Oulu, Oulu, Finland.

SNORING AS A RISK FACTOR FOR HYPERTENSION AND ANGINA PECTORIS

The association of snoring with hypertension and ischaemic heart disease (IHD) was tested by postal questionnaire in a population of 3847 men and 3664 women aged 40-69 years. Hypertension associated highly significantly with snoring, the relative risk (RR) of hypertension between habitual snorers and never snorers being 1.94 in men and 3.19 in women. This association was also found when adjusting for body-mass index. A significant association between angina pectoris and habitual snoring was observed in men (RR = 2.22). In women the relative risk was not significant. An association between habitual snoring and angina pectoris in men was also found after adjusting for hypertension and body-mass index (RR = 2.01, $p<0.01$). The relative risks for myocardial infarction and hospital admission for IHD for habitual snorers were non-significant.

Koskenvuo, M. et al.

The Lancet (I)893-896, 1985.

Other support: The Medical Research Council, Academy of Finland, and the Paavo Nurmi Foundation.

From the Departments of Public Health Science and Neurology, University of Helsinki, Finland.

CANCER STUDIES IN TWINS AND FAMILIES OF TWINS

The study of concordance for cancer in twin pairs can be used to examine the role of heritable factors in cancer. The Finnish Twin Cohort — all like-sexed adult twin pairs born before 1958 in Finland with both co-twins alive in 1967 (n = 17,357 pairs) — was linked to the Finnish Cancer registry data to yield incident cases of cancer up to 1981. Zygosity was determined by the validated questionnaire method in 1975.

A total of 1,112 cases of cancer was found in the twin series up to 1981 among 1,068 twins. Multiple primary cancer was found among 40 persons (3.8%). There were 233 MZ pairs discordant for cancer and 18 pairs in which both members had cancer (probandwise concordance rate = 13.4%). In the DZ pairs there were 478 ciscordant pairs and 34 concordant pairs (probandwise concordance rate 12.5%). When cases of low malignancy were excluded (basal cell carcinoma, *in situ* uterine cervix carcinoma, urinary bladder papilloma, polycythemia vera and myelofibrosis) the figures for MZ pairs were 12 concordant and 202 discordant (rate 10.6%), and for DZ pairs 23 concordant and 413 discordant (rate 10.0%).

The analysis of concordance indicates that overall hereditary factors do not contribute much to the incidence of cancer. It is in accordance with the epidemiologic evidence for the primary role of environmental factors in the etiology of most cancers.

Kaprio, J., *Koskenvuo, M.*, Teppo, L., Langinvainio, K., Pukkala, E., Rita, H., and Sarna, S.

In: *Familial Cancer*. 1st International Research Conference, Basel 1985, pp. 192–194 (Karger, Basel, 1985).

Other support: Sigrid Juselius Foundation.

From the Finnish Twin Cohort Study, Department of Public Health Science, University of Helsinki, and Finnish Cancer Registry, Helsinki, Finland.

ACTIVE PROJECTS

Following is a list of the principal investigators, or institutions, of projects under way or activated in the period since the previous Report, together with the resepective project titles. Completed projects are listed in a later section.

PRINCIPAL INVESTIGATOR OR INSTITUTION	PROJECT TITLE
ROBERT H. ABELES, PH.D. *Professor of Biochemistry*, Brandeis University, Waltham, MA.	Development of elastase inhibitors
LEO G. ABOOD, PH.D. *Professor of Brain Research and Biochemistry*, Center for Brain Research, University of Rochester Medical Center, Rochester, NY.	Nicotine transfer-disposition in liver cells
DOLPH O. ADAMS, M.D., PH.D. *Professor of Pathology*, Duke University Medical Center, Durham, NC.	Role and regulation of protein phosphorylation during microphage activation
KENNETH ADLER, PH.D. *Assistant Professor of Pathology*, University of Vermont College of Medicine, Burlington.	Airway mucin secretion: effects of products from bacteria associated with chronic bronchitis
JOHN J. ALBERS, PH.D. *Research Associate Professor of Medicine*, University of Washington School of Medicine, Seattle.	High density lipoprotein quantitation
HARRY N. ANTONIADES, PH.D. *Professor of Biochemistry*, Harvard University School of Public Health, Boston, MA.	Biosynthesis and processing of PDGF-like polypeptides in human malignant cells in culture
THOMAS M. AUNE, PH.D. *Assistant Professor of Pathology*, The Jewish Hospital of St. Louis, MO.	Biology of the lymphokine, soluble immune response suppressor (SIRS) Interferon-activation of supressor T cell pathways
IRIT AVIRAM, PH.D. *Department of Biochemistry*, The Faculty of Life Sciences, Tel Aviv University, Israel.	Isolation, properties and physiological function of neutrophil cytochrome *b*
BERNARD M. BABIOR, M.D., PH.D. *Professor of Medicine*, New England Medical Center Hospital, Boston, MA.	Studies on the mechanism of activation of the respiratory burst in neutrophils
SAMUEL BALK, M.D., PH.D. *Pathologist*, New England Deaconess Hospital, Boston, MA.	Serum mitogens, hormones, ions, viral transforming genes and tumor reversal in appropriate and autonomous initiation of cell replication

PRINCIPAL INVESTIGATOR OR INSTITUTION	PROJECT TITLE
JOEL S. BENNETT, M.D. *Associate Professor of Medicine*, Hospital of the University of Pennsylvania, Philadelphia.	Characterization of the platelet fibrinogen receptor
RICHARD J. BING, M.D. *Professor of Medicine (emeritus)*, University of Southern California School of Medicine, Los Angeles; *Visiting Associate*, California Institute of Technology; *Director of Experimental Cardiology and Scientific Development*, Huntington Medical Research Institutes, Pasadena, CA.	Coronary spasm; cerebral microcirculation
PHYLLIS B. BLAIR, PH.D. *Professor of Immunology*, University of California, Berkeley.	Regulation of natural killer cell activity
THOMAS R. BROKER, PH.D. *Associate Professor of Biochemistry*, University of Rochester School of Medicine, Rochester, NY.	Cellular transformation by papilloma virus recombinants
DOROTHY L. BUCHHAGEN, PH.D. *Assistant Professor*, State University of New York, Downstate Medical Center, Brooklyn, NY.	Oncogene expression in fetal mouse lung
VINCENZO BUONASSISI, M.D. *Senior Scientist and Deputy Director*, W. Alton Jones Cell Science Center, Inc., Lake Placid, NY.	Heparan sulfate proteoglycans and blood homeostatic mechanisms
JOHN W. BURCH, M.D. *Associate Medical Director*, American Red Cross, Rochester Division, Rochester, NY.	Control of arachidonic acid oxygenation in human platelets
DAVID L. BUSBEE, PH.D. *Professor of Toxicology*, Texas A&M University College of Veterinary Medicine, College Station.	Polynuclear aromatic hydrocarbon transport by serum lipoproteins
EDWARD J. CAMPBELL, M.D. *Assistant Professor of Medicine*, Washington University School of Medicine, St. Louis, MO.	Modulators of inflammatory cell proteolytic activity
LAN BO CHEN, PH.D. *Associate Professor of Pathology*, Dana-Farber Cancer Institute, Boston, MA.	Studies on human oat cell carcinomas
YUAN-TSONG CHEN, M.D., PH.D. *Assistant Professor of Pediatrics*, Duke University Medical Center, Durham, NC.	Recombinant DNA approaches to assess risk for lung cancer

PRINCIPAL INVESTIGATOR OR INSTITUTION	PROJECT TITLE
WILLIAM M. CHILIAN, PH.D. *Assistant Research Scientist*, Cardiovascular Center, University of Iowa College of Medicine, Iowa City.	Pathophysiology of the coronary microcirculation
DOUGLAS BROCK CINES, M.D. *Professor of Medicine* Hospital of the University of Pennsylvania, Philadelphia.	Immune injury of human endothelial cells
CURT I. CIVIN, M.D. *Assistant Professor of Oncology and Pediatrics*, The Johns Hopkins Oncology Center, Baltimore, MD.	Biochemistry and function of human granulopoietic antigens
GARY A. CLAWSON, M.D., PH.D. *Assistant Professor*, University of California, San Francisco.	Nuclear NTPase and selective RNA splicing/transport
BRIAN L. CLEVINGER, PH.D. *Assistant Professor of Biomedical Science*, Washington University of Dental Medicine, St. Louis, MO.	Role of J segment in V segment expression
CHARLES G. COCHRANE, M.D. *Member, Department of Immunopathology*, Scripps Clinic and Research Foundation, La Jolla, CA.	Mediation systems in inflammatory lung disease
ROBERT W. COLMAN, M.D. *Professor of Medicine*, Temple University School of Medicine, Philadelphia, PA.	Initiation of plasma coagulation and kinin forming systems in man
EVA BROWN CRAMER, PH.D. *Associate Professor of Anatomy and Cell Biology*, Downstate Medical Center, Brooklyn, NY.	Studies of inflammation using an *in vitro* model
CARL E. CRUETZ, PH.D. *Assistant Professor of Pharmacology*, University of Virginia School of Medicine, Charlottesville.	Role of protein phosphorylation in nicotine-induced catecholamine release
GIDON CZAPSKI, M.SC., PH.D. *Professor of Physical Chemistry*, The Hebrew University, Jerusalem, Israel.	Role of metal ions on superoxide and Vitamin C toxicity in biological systems
IVAN DAMJANOV, M.D., PH.D. *Professor of Pathology*, Hahnemann University School of Medicine, Philadelphia, PA.	Developmentally pluripotent human lung cancer stem cells
ALBERT B. DEISSEROTH, M.D., PH.D. *Professor of Medicine*, Veterans Administration Medical Center, San Francisco, CA.	Study of altered alpha globin genes in leukemia and solid tumors

PRINCIPAL INVESTIGATOR OR INSTITUTION	PROJECT TITLE
PETER H. DUESBERG, PH.D. *Professor of Molecular Biology*, University of California, Berkeley.	Transforming genes of two acute leukemia viruses
HAROLD F. DVORAK, M.D. *Chief, Department of Pathology*, Beth Israel Hospital, Boston, MA.	Pathogenesis of tumor desmoplasia
V. GENE ERWIN, PH.D. *Professor of Pharmacology; Dean*, University of Colorado School of Pharmacy, Boulder.	Effects of nicotine on neuropeptide secretion by intact mouse brain, a pharmacogenetic study
ALVAN R. FEINSTEIN, M.D. *Professor of Medicine and Epidemiology*, Yale University School of Medicine, New Haven, CT.	Smoking, detection bias and primary lung cancer
THOMAS H. FINLAY, PH.D. *Associate Professor of Obstetrics and Gynecology*, New York University Medical Center, New York.	Structure, properties and regulation of mouse plasma protease inhibitors
PAUL B. FISHER, PH.D. *Senior Research Associate, Department of Microbiology*, Columbia University College of Physicians & Surgeons, New York.	Chemical-viral interactions in cell transformation
JOSEPH A. FONTANA, M.D., PH.D. *Assistant Professor of Medicine and Biochemistry*, West Virginia University Medical Center, Morgantown.	Glycosyltransferases and glycoprotein synthesis in differentiation induced phenotypic reversal of malignancy by retinoic acid cyclic nucleotides and other agents
JUDITH ANN FOSTER, PH.D. *Professor and Chairperson, Department of Biology*, Syracuse University, Syracuse, NY.	Involvement of elastin fibers in lung disease
RICHARD B. FOX, M.D. *Assistant Professor of Pediatrics*, Children's Hospital Corporation, Boston, MA.	Role of glycosaminoglycans in lung edema
IRWIN FRIDOVICH, PH.D. *Professor of Biochemistry*, Duke University Medical Center, Durham, NC.	Control of the biosynthesis of superoxide dismutases
ERROL C. FRIEDBERG, M.D. *Associate Professor of Pathology*, Stanford University, Stanford, CA.	Complementing human cells with cloned yeast DNA repair genes

PRINCIPAL INVESTIGATOR OR INSTITUTION	PROJECT TITLE
KJELL FUXE, M.D. *Professor of Histology*, The Karolinska Institute, Stockholm, Sweden	Nicotine, catecholamines, and neuroendocrine functions
	Smoking, dopamine, neuropeptides and models of Parkinson's disease
JAMES W. GAUBATZ, PH.D. *Assistant Professor of Biochemistry*, University of South Alabama, Mobile.	Direct demonstration of high affinity drug-DNA interactions by restriction enzyme mapping
JACK GAULDIE, PH.D. *Professor of Pathology*, McMaster University, Hamilton, Ontario, Canada	The mast cell in interstitial pulmonary fibrosis
J. BERNARD L. GEE, M.D. *Professor of Medicine*, Yale University School of Medicine, New Haven, CT.	Tissue matrix and phagocyte injury: relative contributions of proteases and oxidants
CHOU ZEN GIAM, PH.D. *Postdoctoral Fellow*, National Institutes of Health, Bethesda, MD.	Immunoglobulin enhancer elements in tissue specific gene expression
JACQUES E. GIELEN, PH.D. *Associate Professor, Laboratory of Medical Chemistry, Toxicology and Hygiene, Institute of Pathology*, University of Liège, Liège, Belgium.	Towards a molecular understanding of mono-oxygenase regulatory mechanisms in animals and man
GORDON NELSON GILL, M.D. *Professor of Medicine*, University of California, San Diego, La Jolla.	Epidermal growth factor receptor gene in epidermoid carcinoma
GABRIEL C. GODMAN, M.D. *Professor of Pathology*, Columbia University College of Physicians & Surgeons, New York.	Cytoskeletal organization of the endothelial cell in regulation of shape contractility and surface movement
WARREN M. GOLD, M.D. *Professor of Medicine*, Cardiovascular Research Institute, University of California, San Francisco.	Effect of ozone on airway mast cells
ALFRED L. GOLDBERG, PH.D. *Professor of Physiology*, Harvard Medical School, Boston, MA.	Selective degradation of damaged cellular proteins
SIDNEY GOLDFISCHER, M.D. *Professor of Pathology*, Albert Einstein College of Medicine, The Bronx, NY.	Extracellular matrix-cytochemistry and ultrastructure
WILLIAM E. GOLDMAN, PH.D. *Assistant Professor of Microbiology and Immunology*, Washington University School of Medicine, St. Louis, MO.	Bordetella pertussis tracheal cytotoxin

PRINCIPAL INVESTIGATOR OR INSTITUTION	PROJECT TITLE
CHARLES S. GREENBERG, M.D. *Assistant Professor of Medicine*, Duke University Medical Center, Durham, NC.	Transglutaminases and atherosclerosis
MARK I. GREENE, M.D., PH.D. *Director of Immunobiology*, University of Pennsylvania, Philadelphia.	Suppressor cells in syngeneic tumor immunity
NOBUYOSHI HAGINO, M.D., PH.D. *Professor of Anatomy*, University of Texas Health Science Center, San Antonio.	Nicotinic receptors of LHRH axon terminals in the median eminence
	Nicotine on prolactin secretion in development
CAROLINE B. HALL, M.D. *Associate Professor of Pediatrics and Medicine*, University of Rochester School of Medicine, Rochester, NY.	Interrelationship of infectious lower respiratory tract disease in infancy, and host and environmental factors to later development of chronic lung disease
LINDA M. HALL, PH.D. *Associate Professor of Genetics and Neuroscience*, Albert Einstein College of Medicine of Yeshiva University, The Bronx, NY.	Genetic differences in nicotine sensitivity in *Drosophila melanogaster* strains
PAUL HAMOSH, M.D. *Associate Professor of Physiology and Biophysics, and Medicine*, Georgetown University Schools of Medicine and Dentistry, Washington, DC.	Cigarette smoke and lipoprotein remodeling by the lung
RONALD G. HARVEY, PH.D. *Professor of Organic Chemistry*, The University of Chicago, IL.	Novel anticarcinogenic coumarins and flavones
ROBERT M. HOFFMAN, PH.D. *Assistant Professor of Pediatrics in Residence*, University of California School of Medicine, La Jolla.	Methionine dependence, methylation and organic transformation
	Regulation of cellular oncogenes
WAYNE HOSS, PH.D. *Associate Professor*, Center for Brain Research, University of Rochester Medical Center, Rochester, NY.	Studies of nicotine interaction with blood cells
RICHARD L. HUGANIR, PH.D. *Assistant Professor*, The Rockefeller University, New York.	The nicotine acetylcholine receptor: regulation by protein phosphorylation
HAROLD P. JONES, PH.D. *Assistant Professor of Biochemistry*, University of South Alabama, Mobile.	Calcium-dependent regulatory proteins and neutrophil activation
MORRIS J. KARNOVSKY, M.B., B.CH. *Shattuck Professor of Pathological Anatomy*, Harvard Medical School, Boston, MA.	The molecular basis of pulmonary surfactant secretion by type II pneumocytes: studies in intact cells and a cell-free system

PRINCIPAL INVESTIGATOR OR INSTITUTION	PROJECT TITLE
SIMON KARPATKIN, M.D. *Professor of Medicine*, New York University Medical Center, New York.	The role of platelets in tumor cell metastases
ROBERT W. KARR, M.D. *Assistant Professor of Medicine*, University of Iowa, Iowa City.	Development and differentiation of normal and leukemic monocytes
SHIRLEY L. KAUFFMAN, M.D. *Professor of Pathology*, State University of New York, Downstate Medical Center, Brooklyn,NY.	Oncogenes in chemical carcinogenesis
INGEGERD M. KEITH, PH.D. *Assistant Professor of Anatomy*, University of Wisconsin School of Veterinary Medicine, Madison.	Lung neuroendocrine cell
HEINZ KOHLER, M.D., PH.D. *Director, Department of Molecular Immunology*, Roswell Park Memorial Institute, Buffalo, NY.	Multi-targeting with hybridomas on tumor cells
MARKKU KOSKENVUO, M.D. *Professor and Chairman, Department of Public Health Science*, University of Helsinki, Helsinki, Finland.	The Finnish Twin Cohort Follow-up Study
ABEL LAJTHA, PH.D. *Director*, New York State Research Institute for Neurochemistry and Drug Addiction, New York.	Genetic basis for nicotine response
E. CLINTON LAWRENCE, M.D. *Assistant Professor of Medicine*, Baylor College of Medicine, Houston, TX.	Effects of cigarette smoking on immunoglobulin production by human bronchial lymphocytes
VALERIE K. LINDGREN, PH.D. *Guest Researcher*, National Cancer Institute, Bethesda, MD.	Viral and cellular factors controlling papillomavirus transcripts
ZVI LIVNEH, PH.D. *Scientist*, The Weizmann Institute of Science, Rehovot, Israel.	Mechanism of S.O.S. error-prone repair
JOSEPH D. LOCKER, M.D., PH.D. *Assistant Professor of Pathology and Biochemistry*, University of Pittsburgh School of Medicine, Pittsburgh, PA.	DNA methylation in neoplasia
RONALD B. LUFTIG, PH.D. *Professor and Head, Department of Microbiology and Immunology*, LSU Medical Center, New Orleans, LA.	Interactions between RNA viruses and chemical carcinogens

PRINCIPAL INVESTIGATOR OR INSTITUTION	PROJECT TITLE
RONALD J. LUKAS, PH.D. *Director, Laboratory of Neurochemistry*, St. Joseph's Hospital and Medical Center, Phoenix, AZ.	Influences of nicotine on neuronal expression of acetylcholine receptors
JAN M. LUNDBERG, M.D. *Assistant Professor of Pharmacology*, Karolinska Institute, Stockholm, Sweden.	Sensory neuropeptides and smoke-induced irritation of the respiratory tract
HENRY T. LYNCH, M.D. *Professor and Chairman, Department of Preventive Medicine and Public Health*, Creighton University School of Medicine, Omaha, NE.	Genetic and biomarker studies of cancers of the respiratory tract, pancreas and urinary bladder
BRUCE A. MACHER, PH.D. *Assistant Professor of Pharmaceutical Chemistry*, University of California, San Francisco.	Chemistry and biology of complex carbohydrates
HOWARD S. MAKER, M.D. *Associate Professor of Neurology*, Mount Sinai School of Medicine, New York.	Nicotine action on brain neurotransmitters and in an animal model of Parkinson's disease
RICHARD A. MARKHAM, M.D. *Assistant Professor of Medicine and of Microbiology and Immunology*, The Jewish Hospital of St. Louis, MO.	T cell-mediated immunity in *Pseudomonas aeruginosa*
WALLACE L. McKEEHAN, PH.D. *Senior Scientist* W. Alton Jones Cell Science Center, Inc., Lake Placid, NY.	Endocrine control of human endothelial cell regeneration
ALAN C. McLAUGHLIN, PH.D *Lecturer in Biochemistry and Biophysics*, University of Pennsylvania School of Medicine, Philadelphia.	Interaction of divalent cations with model and biological membranes
STELLA MITRANI-ROSENBAUM, PH.D. *Professor of Virology*, Hebrew University-Hadassah Medical School, Jerusalem, Israel.	Molecular analysis of human genital papilloma virus
FERID MURAD, M.D., PH.D. *Professor of Medicine and Pharmacology*, Stanford University, and *Chief of Medicine*, Palo Alto V.A. Hospital, Stanford, CA.	Mechanism of nitric oxide activation of guanylate cyclase Role of cyclic GMP in smooth muscle relaxation
CHRISTOPHER MURLAS, M.D. *Assistant Professor of Medicine*, University of Cincinnati, OH.	Electromechanical properties of airway muscle
JAY A. NADEL, M.D. *Professor of Medicine, Physiology and Radiology*, Cardiovascular Research Institute, University of California, San Francisco.	Mechanisms of airway hyperreactivity

PRINCIPAL INVESTIGATOR OR INSTITUTION	PROJECT TITLE
SUSAN NAYLOR, PH.D. *Associate Professor of Human Genetics*, The University of Texas Health Science Center, San Antonio.	Molecular and genetic analysis of small cell lung cancer
DONALD J. NELSON, PH.D. *Associate Professor of Chemistry*, Clark University, Worcester, MA.	Calmodulin interactions with target proteins and synaptic vesicles
STEFAN NIEWIAROWSKI, M.D., PH.D. *Professor of Physiology*, Thrombosis Research Center, Temple University School of Medicine, Philadelphia, PA.	Platelet interaction with fibrinogen and its significance in hemostasis
JANET M. OLIVER, PH.D. *Professor of Pathology*, University of New Mexico School of Medicine, Albuquerque.	Regulation of the membrane oxidase of human polymorphonuclear leukocytes
F. WILLIAM ORR, M.D. *Associate Professor of Pathology*, University of Manitoba, Winnipeg, Manitoba, Canada.	Role of local factors in pulmonary metastasis
YOSHIO OSAWA, PH.D *Head, Department of Endocrine Biochemistry*, Medical Foundation of Buffalo, Buffalo, NY.	Aromatase inhibitors in cigarette smoke and tobacco
BORIS M. PETERLIN, M.D. *Assistant Professor of Medicine*, Section of Rheumatology-Clinical Immunology, University of California School of Medicine, San Francisco.	Biology and molecular biology of the differentiation of a human monocytoid cell line
DENNIS R. PETERSEN, PH.D. *Professor of Pharmacology*, University of Colorado School of Pharmacy, Boulder.	Implementation of the isolated perfused liver to study nicotine metabolism and metabolic interactions
EDGAR PICK, M.D., PH.D. *Professor of Immunology*, Tel Aviv University, Tel Aviv, Israel.	The biochemical basis of enhanced oxygen radical production by lymphokine-activated macrophages
SALVATORE V. PIZZO, M.D., PH.D. *Associate Professor of Pathology*, Duke University Medical Center, Durham, NC.	Protease regulation and cellular metabolism
JULIA M. POLAK, D.SC., M.D. *Senior Lecturer in Histopathology*, Royal Postgraduate Medical School, Hammersmith Hospital, London, England.	Investigation of the role of regulatory peptides in human lung disease
WILLIAM A. PRYOR, PH.D. *Boyd Professor of Chemistry*, Louisiana State University, Baton Rouge.	Free radical chemistry of cigarette smoke

PRINCIPAL INVESTIGATOR OR INSTITUTION	PROJECT TITLE
MICHAEL S. RABSON, M.D. *Research Scientist, Laboratory of Pathology*, National Cancer Institute, Bethesda, MD.	Analysis of sequences required for bovine papilloma virus transformation and autonomous plasmid replication
RAMI RAHAMINOFF, M.D. *Professor of Physiology*; Hebrew University-Hadassah Medical School, Jerusalem, Israel.	Humoral effects of small cell carcinoma of the lung on neuromuscular transmission
TIMOTHY J. REGAN, M.D. *Professor of Medicine; Director, Division of Cardiovascular Diseases*, College of Medicine and Dentistry of New Jersey, New Jersey Medical School, Newark.	Susceptibility of arrhythmias and catecholamine metabolism in chronic smoking animals
JOHN E. REPINE, M.D. *Assistant Director, Webb-Waring Lung Institute; Associate Professor of Medicine*, University of Colorado Health Sciences Center, Denver.	Basic mechanisms of lung injury from inhaled oxidants
ROBERT RESNICK, M.D. *Associate Professor of Reproductive Medicine*, University of California Medical Center, San Diego.	The effect of nicotine on uterine and fetal cardiovascular hemodynamics
VIRGINIA L. RICHMOND, PH.D. *Research Associate*, Pacific Northwest Research Foundation, Seattle, WA.	Elastic fiber microfibrillar protein structure
PETER M. ROSS, PH.D. *Research Associate*, The Rockefeller University, New York.	DNA damage and selective maintenance of animal genes
UNA S. RYAN, PH.D. *Research Professor of Medicine*, University of Miami School of Medicine, Miami, FL.	Interactions of hormones with cells of the pulmonary vascular wall
BRAHMI P. SANI, PH.D. *Head, Protein Biochemistry*, Southern Research Institute, Birmingham, AL.	Selenium-binding proteins
REGINA M. SANTELLA, PH.D. *Associate Professor of Medicine and Environmental Sciences*, Columbia University, New York.	Development of monoclonal antibodies to carcinogen-DNA adducts
B.V. RAMA SASTRY, D.SC., PH.D. *Professor of Pharmacology*, Vanderbilt University School of Medicine, Nashville, TN.	Maternal smoking and blood concentrations of amino acids in umbilical arteries and veins Influence of nicotine on the release of acetylcholine in the human placenta and its implications on the fetal growth

PRINCIPAL INVESTIGATOR OR INSTITUTION	PROJECT TITLE
CHARLES H. SCOGGIN, M.D. *Head, Division of Clinical Applications; Associate Professor of Medicine*, University of Colorado Health Sciences Center, Denver.	The somatic cell genetics of lung cancer
ROBERT E. SCOTT, M.D. *Professor of Pathology*, Mayo Clinic and Foundation, Rochester, MN.	Commitment control and carcinogenesis in normal, preneoplastic and malignant human epithelial cells
HENRY SERSHEN, PH.D. *Research Scientist IV*, Neurochemistry Division, Nathan S. Kline Institute, Ward's Island, New York.	Development of an animal model of Parkinson's disease
ISAIAHU SHECHTER, PH.D. *Senior Lecturer in Biochemistry*, The George S. Wise Faculty for Life Sciences, Tel Aviv University, Tel Aviv, Israel.	Effect of thiols and disulfides on cholesterol metabolism
ROBERT J. SKLAREW, PH.D. *Research Associate Professor of Pathology*, New York University Research Service, Goldwater Memorial Hospital, Roosevelt Island, NY.	Cytokinetics of heteroploid subpopulations by imaging
KENDALL A. SMITH, M.D. *Professor of Medicine*, Dartmouth Medical School, Hanover, NH.	Dissection of the eukaryotic DNA replication pathway
STEVEN S. SMITH, PH.D. *Assistant Research Scientist*, Beckman Research Institute of the City of Hope, Duarte, CA.	Selectivity of DNA methylation in normal and oncogenically transformed cells
TIMOTHY A. SPRINGER, PH.D. *Assistant Professor of Pathology; Chief, Laboratory of Membrane Immunochemistry*, Dana-Farber Cancer Institute, Boston, MA.	Studies of macrophage subpopulations and differentiation using monoclonal antibodies
ERIC J. STANBRIDGE, PH.D. *Associate Professor of Microbiology*, University of California, Irvine.	Transfer of specific individual human chromosomes to recipient cells
NORMAN C. STAUB, M.D. *Professor of Physiology*, Cardiovascular Research Institute, University of California, San Francisco.	Alveolar-airway barrier permeability to liquid and macromolecules in dog and sheep lung
DANIEL STEINBERG, M.D., PH.D. *Professor of Medicine; Head, Division of Metabolic Disease*, The University of California at San Diego, La Jolla.	Arterial degradation of low density lipoproteins *in vivo*

PRINCIPAL INVESTIGATOR OR INSTITUTION	PROJECT TITLE
THOMAS P. STOSSEL, M.D., *Chief, Medical Oncology Unit*, Massachusetts General Hospital, Boston.	Functional anatomy of the lung macrophage
FLEUR L. STRAND, PH.D. *Professor of Biology*, New York University, New York.	Prenatal and postnatal effects of nicotine and ACTH peptides on neuromuscular development and motor behavior in rats
D. LANSING TAYLOR, PH.D. *Professor of Biology*, Carnegie-Mellon University, Pittsburgh, PA.	Chemotaxis of macrophages
JOSEPH CHARLES TAYLOR, PH.D. *Associate Research Scientist*, City of Hope Research Institute, Duarte, CA.	Ceruloplasmin abnormality in chronic obstructive pulmonary disease
JOHN A. THOMPSON, PH.D. *Associate Professor of Pharmaceutical Chemistry*, University of Colorado School of Pharmacy, Boulder.	Chromatographic separation and comparative metabolism of d- and l-nicotine
WAYNE M. TREBBIN, M.D. *Nephrologist*, Roger Williams General Hospital, Providence, RI.	The effects of renal function on nicotine metabolism
EMIL R. UNANUE, M.D. *Chairman and Professor, Dept. of Pathology*, Washington University School of Medicine, St. Louis, MO.	Physiopathology of normal and activated macrophages
HAROLD E. VARMUS, M.D. *Professor of Microbiology and Immunology*, University of California, San Francisco.	Functional analysis of cellular oncogenes activated during tumorigenesis
HELEN VAN VUN ..IS, PH.D. *Professor of Biochemistry*, Brandeis University, Waltham, MA.	Purification and properties of a soluble NAD(P) glycohydrolase isolated from the sponge, *M. prolifera*
ZBIGNIEW WALASZEK, PH.D. *Research Scientist*, Ohio State University, Columbus.	Potential for lung cancer prevention by inhibiting de-glucuronidation
EVELYN WALDSTEIN, PH.D. *Senior Lecturer*, Department of Biochemistry, Tel Aviv University, Tel Aviv, Israel.	Repair activities for O^6-methylguanine DNA adducts in human lymphocytes of smokers *vs.* nonsmokers
PETER N. WALSH, PH.D. *Professor of Medicine*, Temple University School of Medicine, Philadelphia.	Interaction of platelets with coagulation factors IX and X
PETER A. WARD, M.D. *Professor and Chairman, Department of Pathology*, The University of Michigan, Ann Arbor.	Oxygen-derived free radicals, immune complexes and tissue injury

PRINCIPAL INVESTIGATOR OR INSTITUTION	PROJECT TITLE
GEORGE WEINBAUM, PH.D. *Assistant Chairman for Research, Department of Medicine*, The Graduate Hospital, Philadelphia.	The role of peptide methionine sulfoxide reductase in human lungs: a possible defense against protein oxidation and elastin degradation in smokers
SAMUEL B. WEISS, PH.D. *Professor of Biochemistry and Microbiology*, The University of Chicago, Chicago.	Sequence modifications in viral DNA by benzo(α)pyrene metabolites
HOWARD G. WELGUS, M.D. *Assistant Professor of Medicine*, Jewish Hospital at Washington University Medical Center, St. Louis, MO.	Human macrophage collagenase and collagenase inhibitor
MICHAEL J. WELSH, M.D. *Assistant Professor of Medicine*, University of Iowa College of Medicine, Iowa City.	Mechanisms controlling ion transport in airway epithelia
ÅKE WENNMALM, M.D. *Professor and Chairman, Department of Clinical Physiology*, University of Gothenburg, Gothenburg, Sweden.	Nicotine as inhibitor of prostaglandin formation: localization of the inhibitory step and characterization of the cardiovascular implications
PAUL V. WOOLEY, III, M.D. *Professor of Medicine and Pharmacology*, Georgetown University Medical Center, Washington, D.C.	Effects of chemical carcinogens upon gene loci in the pancreas
STANLEY YACHNIN, M.D. *Professor of Medicine and Chief, Section of Hematology/Oncology*, The University of Chicago Medical Center, Chicago.	Models for the pathogenesis of atherosclerosis: A) biological effects of oxygenated sterol compounds, B) mevalonic acid and cholesterol biosynthesis and the biosynthesis and regulation of cell growth.
DONALD A. YOUNG, M.D. *Professor of Medicine*, University of Rochester, Rochester, NY.	Papilloma virus proteins and cell transformation

COMPLETED PROJECTS

Following is a list of the principal investigators, or institutions, of projects that have been completed prior to the period covered in this Report. Several of the individuals named are deceased. The titles and affiliations listed were those in effect at the time the work was in progress.

MARIO D. ACETO, PH.D.
Associate Professor of Pharmacology, Medical College of Virginia, Virginia Commonwealth University, Richmond.

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Associate Clinical Professor of Medicine, University of California Medical Center, Los Angeles.

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Professor of Physiology, Boston University School of Medicine, Boston, MA.

D. MURRAY ANGEVINE, M.D.
University of Wisconsin School of Medicine, Madison.

JOSEPH C. ARCOS, D.Sc.
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ALAN K. ARMITAGE, PH.D.
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Professor of Pharmacology, University of Pennsylvania School of Medicine, Philadelphia.

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LESLIE BAER, M.D.
Associate Professor of Medicine, Columbia University College of Physicians & Surgeons, New York, NY.

OSCAR J. BALCHUM, PH.D.
Hastings Professor of Medicine, University of Southern California School of Medicine, Los Angeles.

FREDERIK B. BANG, M.D.
Professor and Chairman, Department of Pathobiology, The Johns Hopkins University School of Hygiene and Public Health, Baltimore, MD.

A. CLIFFORD BARGER, M.D.
Robert Henry Pfeiffer Professor of Physiology, Harvard Medical School, Boston, MA.

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Associate Professor of Medicine, The Johns Hopkins University School of Medicine, Baltimore, MD.

T. C. BARNES, D.Sc.
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BENJAMIN BELL, M.D.
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SAMUEL BELLET, M.D.
Director, Division of Cardiology, Philadelphia General Hospital, Philadelphia, PA.

BARUJ BENACERRAF, M.D.
Fabyan Professor and Chairman, Department of Pathology, Harvard Medical School, Boston, MA.

WILLIAM F. BENEDICT, M.D.
Assistant Professor of Pediatrics, University of Southern California School of Medicine, Division of Hematology and Medical Genetics, Children's Hospital of Los Angeles, Los Angeles.

BARBARA J. van den BERG, M.D.
Research Pediatrician, Adjunct Professor in Biostatistics, University of California School of Public Health, Oakland.

JOHN A. BEVAN, M.D.
Professor of Pharmacology, University of California School of Medicine, Los Angeles.

BUDHDEV BHAGAT, PH.D.
Professor of Physiology, Saint Louis University School of Medicine, St. Louis, MO.

CESARE BIANCIFIORI, M.D.
Division of Cancer Research, University of Perugia, Perugia, Italy.

HYLAN A. BICKERMAN, M.D., *Assistant Professor of Medicine*, and ALVAN L. BARACH, M.D., *Consultant in Medicine*, Columbia University College of Physicians & Surgeons, Goldwater Memorial Hospital, New York, NY.

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DEBAJIT K. BISWAS, PH.D., D.SC.
Associate Professor of Oral Biology, Laboratory of Pharmacology, Harvard School of Dental Medicine, Boston, MA.

IRA B. BLACK, M.D.
Professor and Chief, Division of Developmental Neurology, Cornell University Medical College, New York, NY.

FRED G. BOCK, PH.D.
Associate Cancer Research Scientist, Biological Station, Roswell Park Memorial Institute, Springville, NY.

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Head, Department of Chemistry and Biochemistry, Spindletop Research Center, Lexington, KY.

JAMES F. BONNER, PH.D.
Professor of Biology, California Institute of Technology, Pasadena.

WALTER M. BOOKER, PH.D.
Professor and Head, Department of Pharmacology, Howard University, Washington, DC.

FRANÇOIS M. BOOYSE, PH.D.
Senior Investigator, Michael Reese Research Foundation, Chicago, IL.

RAYMOND BOSSE, PH.D.
Associate Director, Normative Aging Study, Veterans Administration Outpatient Clinic, Boston, MA.

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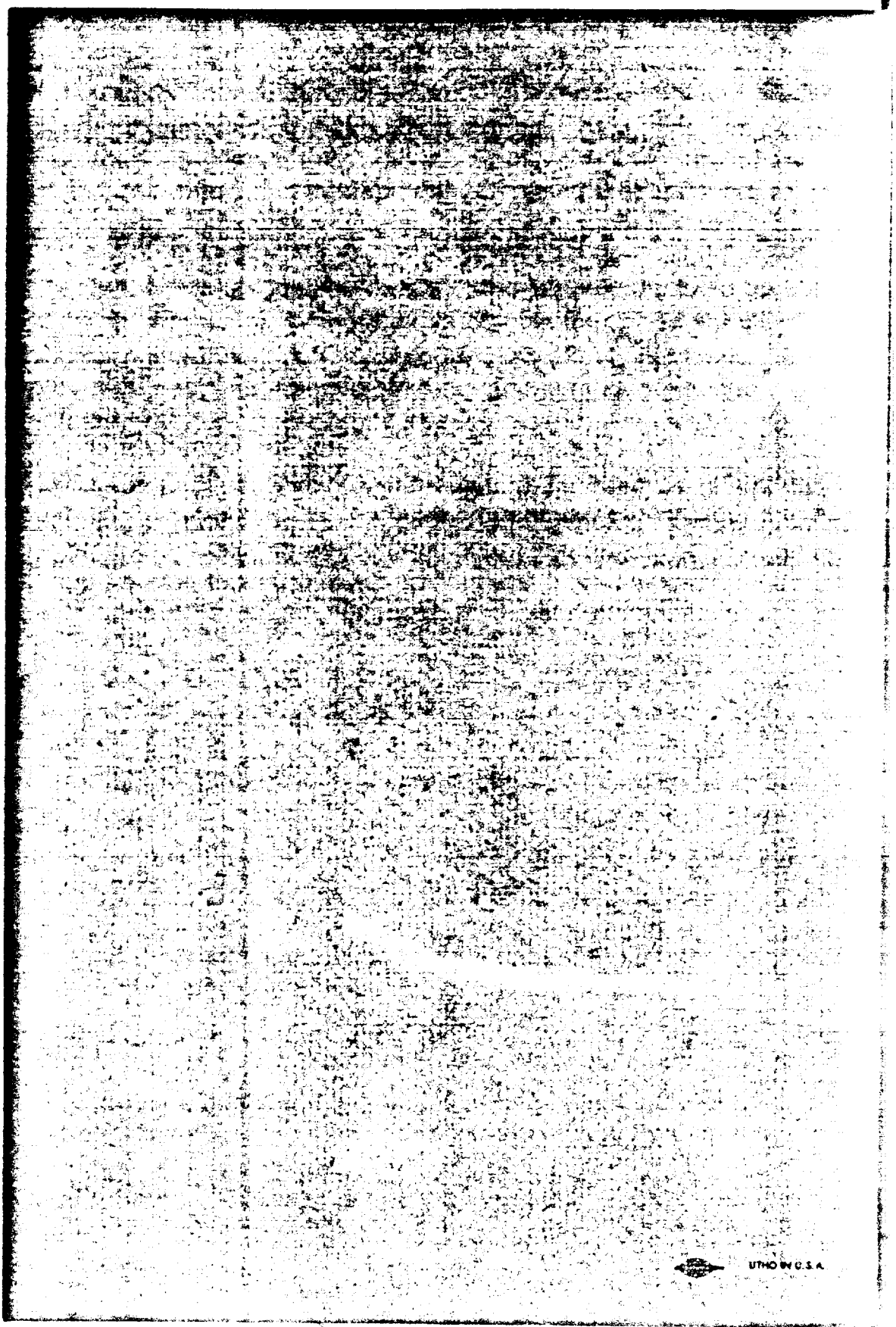

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